Soilborne Biopesticides

Mechanisms, Strengths, Limitations, and How to Improve Them

Brian B. McSpadden Gardener

Professor, Department of Plant Pathology
Director, Organic Food Farming Education & Research Program
mcspadden-garden.1@osu.edu

The Ohio State University
College of Food, Agricultural, and Environmental Sciences

Outline

- Soilborne Biopesticides
  - Types, Mechanisms, Applications
- Strengths and Limitations Today
  - Mitigation and Trade-offs
- Challenges in Product Development
  - The four C’s and How to achieve them
- Future Research Directions
  - Applying “Omics” and the Phytobiome Perspective

Outline

- Soilborne Biopesticides
  - Types, Mechanisms, Applications
- Strengths and Limitations Today
  - Mitigation and Trade-offs
- Challenges in Product Development
  - The four C's and How to achieve them
- Future Research Directions
  - Applying "Omics" and the Phytobiome Perspective

Types of Soilborne Biopesticides
by Active Ingredients

- Plant Incorporated Protectants
  - *Cry* genes (Lepidoptera)
  - Chitinase genes (Fungi)
  - Resistance and effector genes (Diverse targets)

- Hyperparasitic microbial specialists
  - *Coniothyrium minitans* (Sclerotinia)
  - *Pastueria* (RKN)
  - Entomopathogenic nematodes (Grubs)
  - *Sclerotinia minor* (Dandelion and some other dicots)

Types of Soilborne Biopesticides
by Active Ingredients

- Multifactorial microbial generalists
  - *Trichoderma* spp. (Diverse targets)
  - *Bacillus* sp. (Diverse targets)
  - *Streptomyces lydicus* (Diverse targets)

- Biochemical Co-formulates
  - Antibiotic-containing fermentation products (Diverse targets)
  - Plant and seaweed extracts (Plant targets)
  - EPA List4a biochemicals (Diverse targets)

Mechanisms

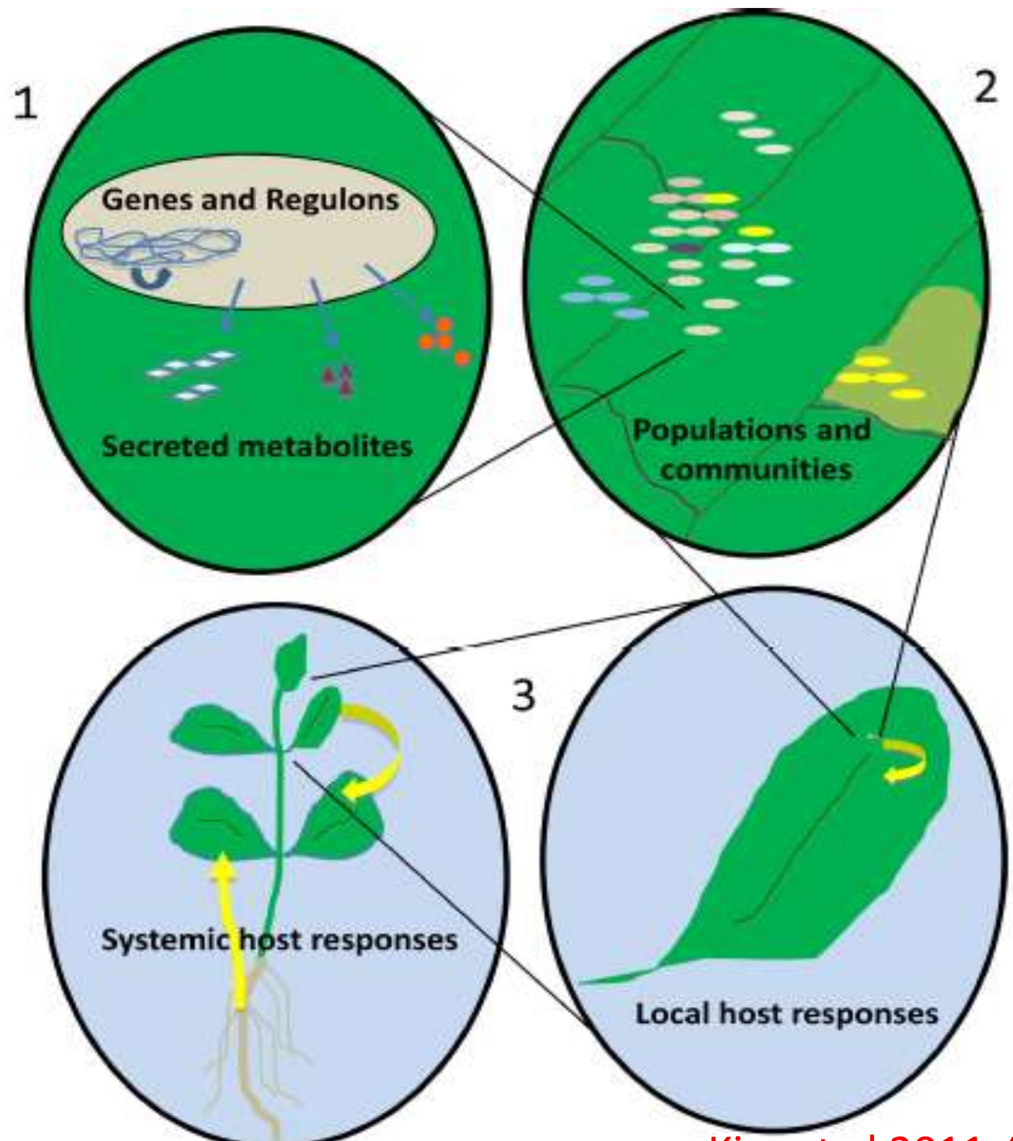
Continuum of Direct to Indirect

Type	Mechanism	Examples
Direct antagonism	Hyperparasitism/predation	Lytic/some nonlytic mycoviruses *Ampelomyces quisqualis* *Lysobacter enzymogenes* *Pasteuria penetrans* *Trichoderma virens*
Mixed-path antagonism	Antibiotics	2,4-diacetylphloroglucinol Phenazines Cyclic lipopeptides
	Lytic enzymes	Chitinases Glucanases Proteases
	Unregulated waste products	Ammonia Carbon dioxide Hydrogen cyanide
	Physical/chemical interference	Blockage of soil pores Germination signals consumption Molecular cross-talk confused
Indirect antagonism	Competition	Exudates/leachates consumption Siderophore scavenging Physical niche occupation
	Induction of host resistance	Contact with fungal cell walls Detection of pathogen-associated, molecular patterns Phytohormone-mediated induction

Pal and McSpadden Gardener 2006 *Plant Health Instructor*

Mechanisms

Multifactorial Nature



Kim et al 2011 *Appl. Environ. Microbiol.*

Applications
Inoculative Approaches

- Seed treatments
  - Applications pre bagging (1 to 18 month)
  - Applications closer to planting (<1 month)

- In hopper/at planting treatments
  - In the hopper (flowable powder, liquid)
  - Transplant dips (liquids/powders)

Applications

Inundative Approaches

- In furrow/potting mix incorporation
  - Flexible rate at planting/ potting (on site)
  - Controlled rate pre-bagging (6 – 24 month)

- Post planting drenches/fertigation
  - Blending with fertility and/or pesticides (liquids)
  - Controlled frequency and rates (alternate, weekly)

Outline

- Soilborne Biopesticides
  - Types, Mechanisms, Applications
- Strengths and Limitations Today
  - Mitigation and Trade-offs
- Challenges in Product Development
  - The four C's and How to achieve them
- Future Research Directions
  - Applying "Omics" and the Phytobiome Perspective

Strengths and Limitations By Type

- PIPs
  - Strengths
    - Whole plant protection
    - Potentially tissue specific and/or inducible
  - Limitations
    - Limited control over expression levels
      - Mitigated by selecting and propagating constitutive expressers that balance need for activity with yield drag
    - Rapid resistance development in the absence of refuges
      - Mitigated with refuges and mixed cultivar plantings
    - Not compliant with certified organic agriculture
      - Mitigated by application to fiber, fuel, and conventional feed and food crops
    - Reduction in beneficial fungal colonization
      - Mitigated by inoculation and/or supplemental inputs

Strengths and Limitations By Type

- Hyperparasitic and Competitive specialists
  - Strengths
    - Specificity for target pest/pathogen
    - Lowers pathogen inoculum if persistent
    - Can be partially curative
  - Limitations
    - Requires high inoculum / endemic disease pressure to be valuable
      - Mitigated by applying only with high disease/pest pressure or persistent colonization of plant root zone
    - Must be compatible with full package of control methods
      - Mitigated with appropriate practices and inputs

Strengths and Limitations By Type

- Microbial generalists
  - Strengths
    - Provide broad spectrum protection against seedling diseases
    - Diverse active ingredients available with multiple modes of action
  - Limitations
    - Low activity per CFU and low control over population size
      - Mitigated by high inoculum rates or selecting for stable colonizers
    - Non-spore formers have limited viability
      - Mitigated by refrigeration or on site culturing

Strengths and Limitations By Type

- Biochemical co-formulates
  - Strengths
    - Can provide broad spectrum protection
    - Diverse actives with different modes of action
    - Some act as biostimulants of plant growth
    - Don’t have to declare activity on label
  - Limitations
    - Limited activity of EPA List4A ingredients
      - Mitigated by complementation with multiple materials
    - Inundative applications may lead to resistance
      - Mitigated by mixing or alternating actives/MOA

Strengths and Limitations By Application

- Seed treatments
  - Strengths
    - Convenient for diverse end users
    - Can use all Types of active ingredients
  - Limitations
    - Primarily targets early season protection
      - Mitigated by selecting for good root colonizers
    - Limited volume of application
      - Mitigated by seed coatings and pelleting

Strengths and Limitations By Application

- In hopper/at planting treatments
  - Strengths
    - Allows for “last minute” addition to protect against unexpected stresses
    - Supports more controlled delivery of “less durable” actives
  - Limitations
    - Requires additional handling and thoughtful timing by grower
      - Mitigated by automating on-site treatment and clear label instructions

Strengths and Limitations By Application

- In furrow/potting mix incorporation
  - Strengths
    - Inundative applications possible in a spatially-defined root zone
    - Compatible with all Types of active ingredients
  - Limitations
    - Heterogeneity of soils and potting mixes limits control provided by live microbials
      - Mitigated by high inoculation rates, selecting for good rhizosphere colonists, and physiological priming

Strengths and Limitations By Application

- Post-planting drenching and chemifertigation
  - Strengths
    - Exquisite control of timing and rate
    - May be compatible with all Types of active ingredients
  - Limitations
    - Limited to irrigated and/or hydroponic production
      - Mitigated by carefully identifying customers with root disease and pest problems
    - Needs to be compatible with other chemistries used
      - Mitigated by alternating applications and proper flushing/cleaning

Outline

- Soilborne Biopesticides
  - Types, Mechanisms, Applications
- Strengths and Limitations Today
  - Mitigation and Trade-offs
- Challenges in Product Development
  - The four C's and How to achieve them
- Future Research Directions
  - Applying "Omics" and the Phytobiome Perspective

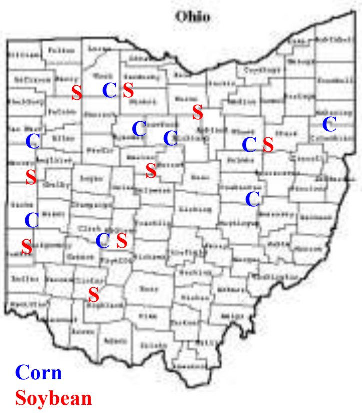
Challenges in Product Development

The Four C's

- Compatibility
  – Need to "fit" into established production and distribution systems
- Complementarity
  – Need to combine with different crop species, chemicals, and other inputs
- Convenience
  – Need to minimally alter grower practice
- Cost Effectiveness
  – Need to provide return on investment for manufacturer, distributer, and grower

An Example For Perspective

phlD+/DAPG-producing *Pseudomonas*



- Biogeography studies revealed
  - 75% of corn roots naturally colonized
  - <45% of soybean roots colonized, but more abundant on colonized soy
  - The D, S, and A types of this bacteria dominant in Ohio fields
  - Natural populations associated with improvements in stand/yields

- Characterization studies revealed
  - Diverse Ohio isolates were capable of inhibiting pathogens of corn and soy *in vitro*
  - Microbial inoculants were formulated as pre plant seed treatments for field testing

McSpadden Gardener et al. 2005 *Phytopathology*

Rotenberg et al 2007 *Phytopathology*

Figure 1: Inoculating seed with DAPG-producing *Pseudomonas* strain for yield enhancement.

Field Testing of Seed Treatments

phlD+ Pseudomonas example

Table 1: Effects of inoculation of soybean seeds with DAPG-producing pseudomonads on organic farms.

Site	Cultivar	Treatment	DAPG-producers log cells / gram root	Yield bu/A
1A	Kottman	Treated	4.3	44.3
		Untreated	3.6	42.8
1B	Kottman	Treated	4.3	28.7
		Untreated	3.3	24.1
2	Vinton	Treated	5.3	38.7
		Untreated	4.6	35.0
3	Vinton	Treated	5.1	29.2
		Untreated	4.4	28.7
All sites combined		Treated	4.8	35.2
		Untreated	4.2	32.6

Over >12 site years, increases in yields on organic farms were comparable to applications to conventional land (+4-6%)

McSpadden Gardener et al. 2006a,b *Biological and Cultural Tests*

Value Proposition
for the Grower Customer

- Seed treatments efficacy assumptions
  - 2 to 5% yield increases on average
  - 3 of 5 years give positive responses
  - similar for chemical and biological treatments
- 0.6 – 1.4 bu / A x $7-$14 / bu = $4 - $20 / A gross return for treatment
- Seed treatments cost $1 to $5 / A
- ROI of 4:1 is typical target for the industry

Problems with *phlD+* Pseudomonads

With Reference to the Four C's

- Compatibility
  - Reasonable RT shelf life, but less durable formulations than spore formers
- Complementarity
  - Tolerates most in furrow fertilizers and some chemicals but more sensitive to chemical stresses than dormant spore formers
- Convenience
  - Works with on seed drip but, with direct seed treatment, requires farmers to treat seed within 48 hrs of planting
- Cost Effectiveness
  - Initially comparable, but on site fermentation may significantly reduce producer and distributor costs

So What are the Options To Achieve The Four C’s?

For Any Microbial Biopesticide

- Compatibility
- Complementarity
- Convenience
- Cost Effectiveness

Options To Achieve The Four C's

Requiring to Plant Pathology Research

- Cost Effectiveness
  - Define the need
    - Quantify risk and loss potential for particular diseases/complexes
    - Identify most susceptible production systems
  - Improve performance of products
    - Discover new and better active ingredients
    - Improve formulation and delivery
  - Minimize production and distribution costs

- Convenience
  - Educate growers when differential handling is required
  - Simplify differential handling
  - Work only with "tough" formulations

Options To Achieve The Four C's

Requiring to Plant Pathology Research

- Compatibility
  - Focus on input substitution
  - Select/breed microbes for crop/system specific colonization
  - Evaluate effects on crop health under field relevant conditions
  - Integrate products with new equipment packages
    - Integrate with on seed   off set variable in furrow applicators
    - Assess value proposition of precision application

- Complementarity
  - Increase tolerance to other chemicals being applied
  - Separate applications in time or space
  - Evaluate efficacy of input "packages"
    - Identify conditions under which mixtures provide better control
    - Model plant health responses to different microbial, chemical, and biochemical combinations

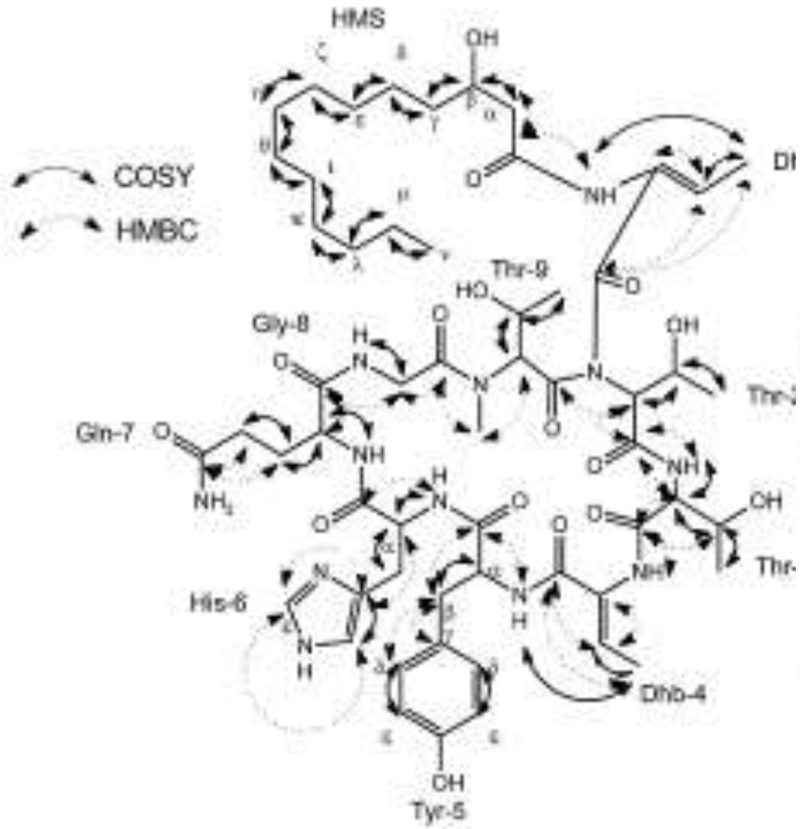
Outline

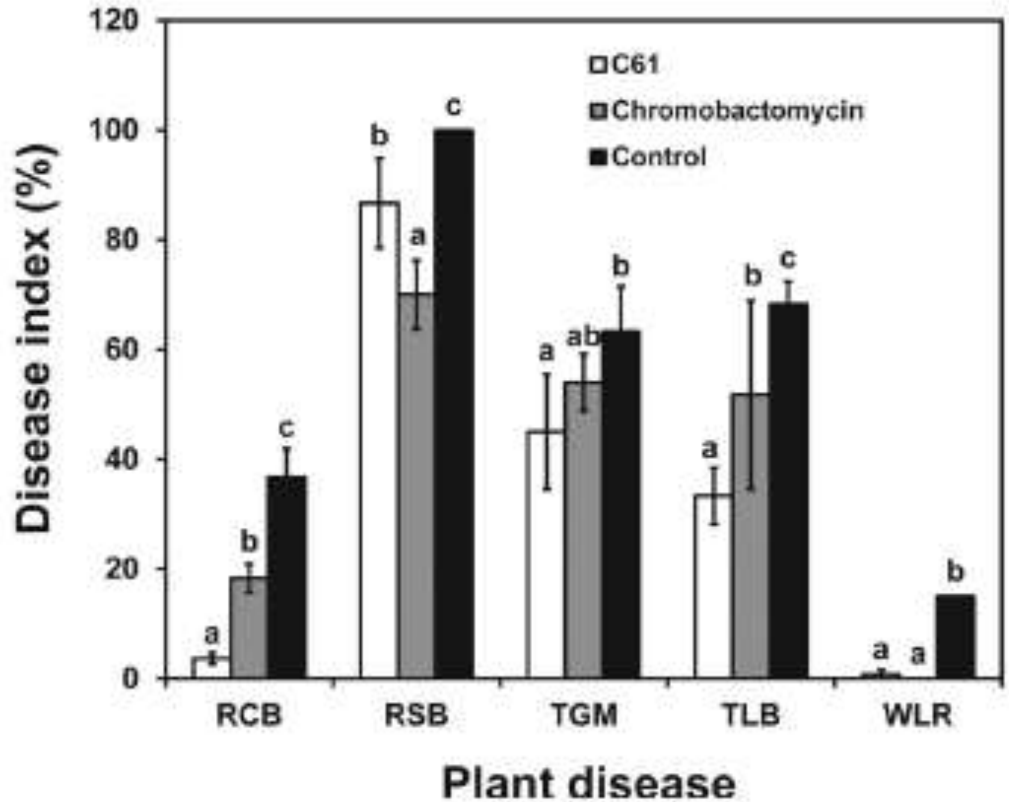
- Soilborne Biopesticides
  - Types, Mechanisms, Applications
- Strengths and Limitations Today
  - Mitigation and Trade-offs
- Challenges in Product Development
  - The four C's and How to achieve them
- Future Research Directions
  - Applying "Omics" and the Phytobiome Perspective

Identifying New Metabolites

Genomics: Pathways used for target selection

Metabolomics: MS and NMR used to characterize new actives





Kim et al 2014 *Molec. Plant Pathol.*

Defining Environmental Constraints

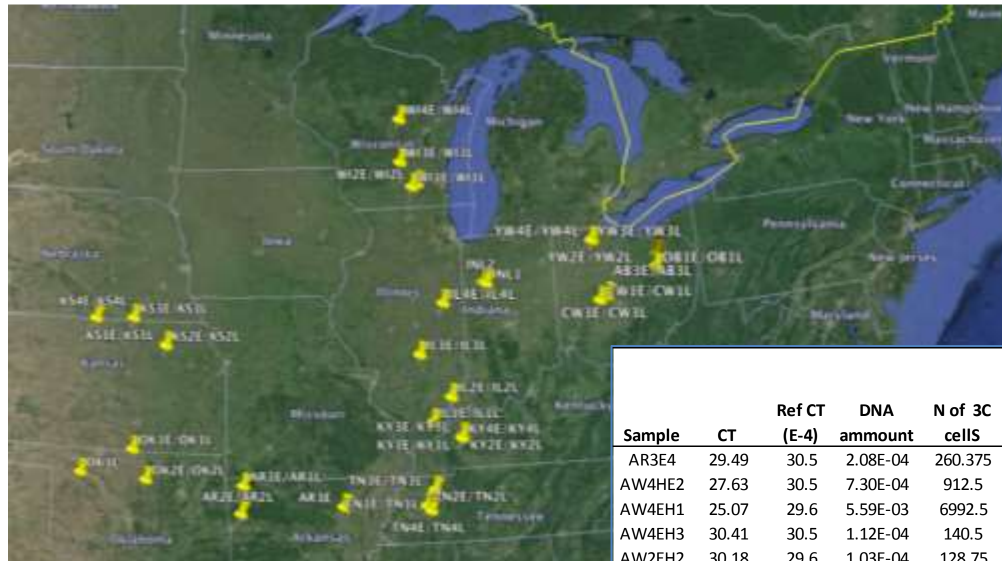
Genomics: Rapid development of qPCR assay to define biogeography of native biocontrol populations and …

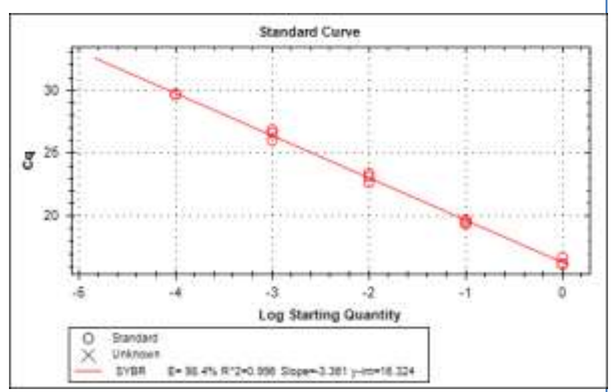
DNA extraction

Dilution 1:20

Screening with Traditional PCR

Quantification qPCR





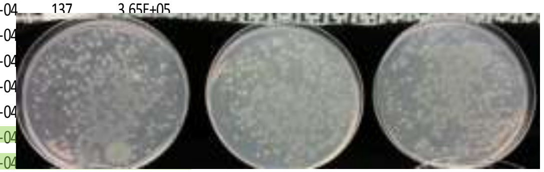
Sample	CT	Ref CT (E-4)	DNA ammount	N of 3C cellS	N of 3C cellS / g of wheat heads
AR3E4	29.49	30.5	2.08E-04	260.375	6.94E+05
AW4HE2	27.63	30.5	7.30E-04	912.5	2.43E+06
AW4EH1	25.07	29.6	5.59E-03	6992.5	1.86E+07
AW4EH3	30.41	30.5	1.12E-04	140.5	3.75E+05
AW2EH2	30.18	29.6	1.03E-04	128.75	3.43E+05
CW1EH3	30.15	30.5	1.34E-04	167.75	4.47E+05
YW1EH3	29.55	29.6	1.68E-04	209.75	5.59E+05
IL3E3	27.57	28.6	1.45E-04	181.75	4.85E+05
KS3E1	30.5	30.5	9.67E-05	120.9	3.22E+05
KY1E4	30.45	30.5	1.10E-04	137	3.65E+05
KY2E3	30.26	30.5	1.25E-04	155.75	4.15E+05
KY3E3	27.89	28.6	1.13E-04	141.375	3.77E+05
KY3E4	26.73	28.6	2.80E-04	349.375	9.32E+05
KY4E3	30.5	30.5	1.02E-04	127.375	3.40E+05
KY4E4	27.73	30.5	6.82E-04	852.5	2.27E+06
NC3E2	29.46	29.8	4.69E-04	586.25	1.56E+06
NC3E3	29.71	30.5	1.80E-04	224.5	5.99E+05
OK1E1	30.5	30.5	1.02E-04	127.875	3.41E+05
OK2E1	29.54	29.6	1.69E-04	211.75	5.65E+05
OK2E4	29.34	29.6	1.99E-04	248.375	6.62E+05
TN1E4	27.71	28.6	1.30E-04	162.375	4.33E+05
TN2E1	29.32	29.8	5.26E-04	657.5	1.75E+06
TN2E3	28.79	29.8	8.00E-04	1000	2.67E+06
WI2E2	28.25	28.6	8.55E-05	106.8625	2.85E+05
WI3E4	29.41	29.6	1.88E-04	235.125	6.27E+05
WI4E4	30.64	30.5	9.64E-05	120.475	3.21E+05

Defining Environmental Constraints

…. allow for marker assisted selection of novel variants to identify superior agents

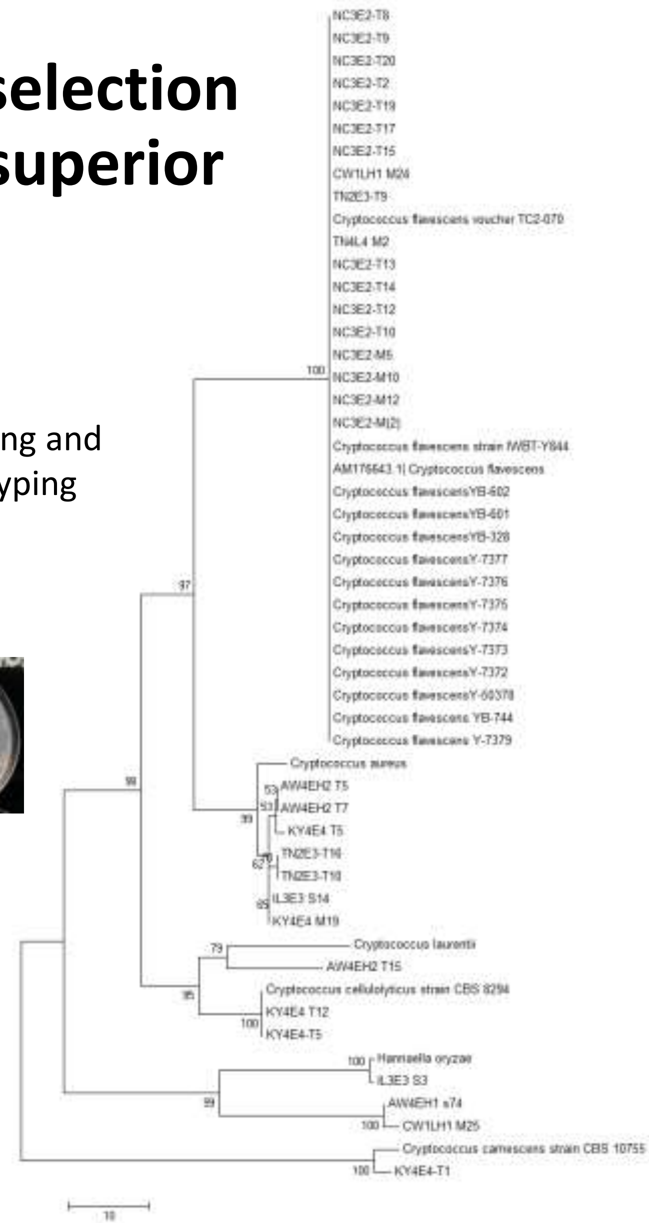
Washing by vortexing and centrifugation

Sample	CT	Ref CT (E-4)	DNA ammount	N of 3C cellS	N of 3C cellS / g of wheat heads
AR3E4	29.49	30.5	2.08E-04	260.375	6.94E+05
AW4HE2	27.63	30.5	7.30E-04	912.5	2.43E+06
AW4EH1	25.07	29.6	5.59E-03	6992.5	1.86E+07
AW4EH3	30.41	30.5	1.12E-04	140.5	3.75E+05
AW2EH2	30.18	29.6	1.03E-04	128.75	3.43E+05
CW1EH3	30.15	30.5	1.34E-04	167.75	4.47E+05
YW1EH3	29.55	29.6	1.68E-04	209.75	5.59E+05
IL3E3	27.57	28.6	1.45E-04	181.75	4.85E+05
KS3E1	30.5	30.5	9.67E-05	120.9	3.22E+05
KY1E4	30.45	30.5	1.10E-04	137	3.65E+05
KY2E3	30.26	30.5	1.25E-04	[illegible]	[illegible]
KY3E3	27.89	28.6	1.13E-04	[illegible]	[illegible]
KY3E4	26.73	28.6	2.80E-04	[illegible]	[illegible]
KY4E3	30.5	30.5	1.02E-04	[illegible]	[illegible]
KY4E4	27.73	30.5	6.82E-04	[illegible]	[illegible]
NC3E2	29.46	29.8	4.69E-04	[illegible]	[illegible]
NC3E3	29.71	30.5	1.80E-04	224.5	5.99E+05
OK1E1	30.5	30.5	1.02E-04	127.875	3.41E+05
OK2E1	29.54	29.6	1.69E-04	211.75	5.65E+05
OK2E4	29.34	29.6	1.99E-04	248.375	[illegible]
TN1E4	[illegible]	[illegible]	[illegible]	[illegible]	4.33E+05
TN2E1	[illegible]	[illegible]	[illegible]	[illegible]	1.75E+06
TN2E3	[illegible]	[illegible]	[illegible]	1000	2.67E+06
WI2E2	[illegible]	[illegible]	[illegible]	106.8625	2.85E+05
WI3E4	[illegible]	[illegible]	[illegible]	235.125	6.27E+05
WI4E4	[illegible]	[illegible]	[illegible]	120.475	3.21E+05



Isolation

Genotyping and Phenotyping



Rotondo and Rong et al. *In Preparation*

Other Targets of "Omic" Research

- Identify new plant genes responsive to (pathogenic and beneficial) microbial colonization
- Define responses of microbes and plants to co-formulates and other environmental parameters
- Identify conditions leading to effective colonization and biofilm formation on seeds, roots and soil constituents

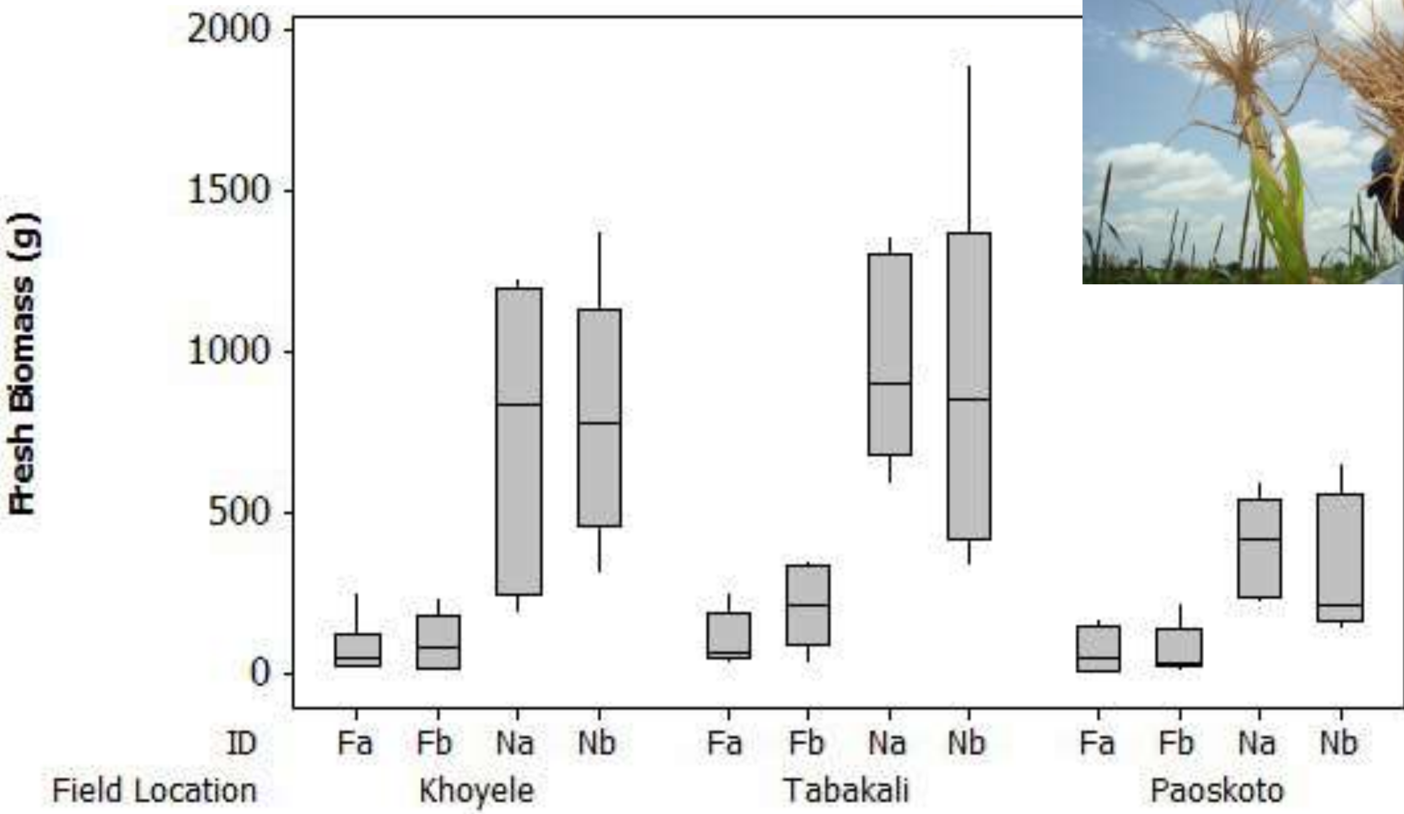
Other Targets of “Omic” Research

- Identify new plant genes responsive to (pathogenic and beneficial) microbial colonization
- Define responses of microbes and plants to co-formulates and other environmental parameters
- Identify conditions leading to effective colonization and biofilm formation on seeds, roots and soil constituents

Goal: Disentangle the relationships between soil, plant, and microbial community variables that all affect plant health

Profiling the Phytobiome

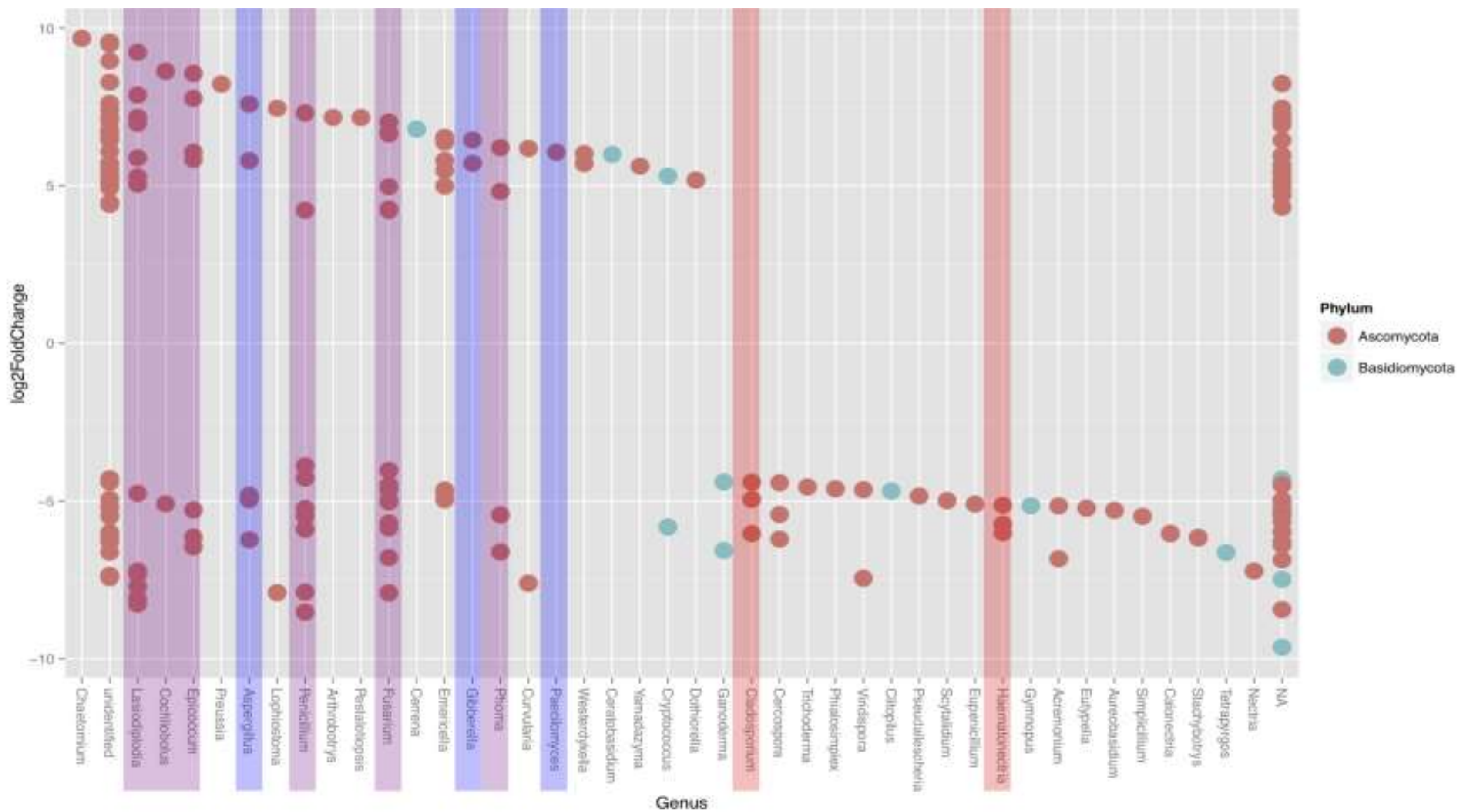
Metagenomics and HTS: Characterize functional gradients…



Debenport et al. *In Preparation*

Profiling the Phytobiome

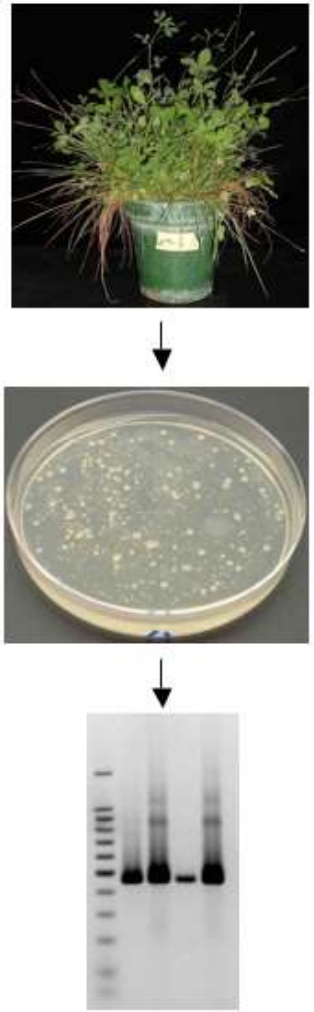
…to associate *multiple* microbial populations with the emergent properties of plant health and productivity

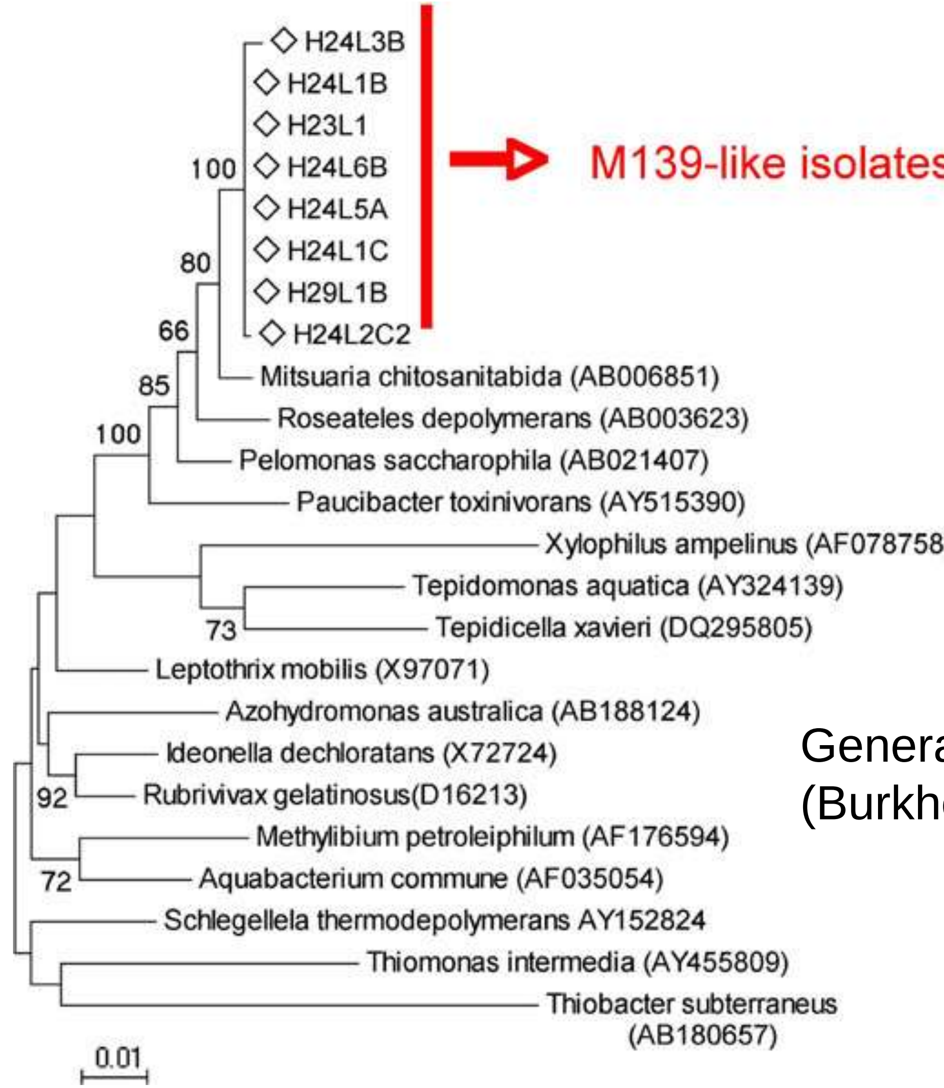


Debenport et al. *In Preparation*

Community Profiling and Marker Assisted Selection

Recovery of a soilborne *Mitsuaria* sp. …

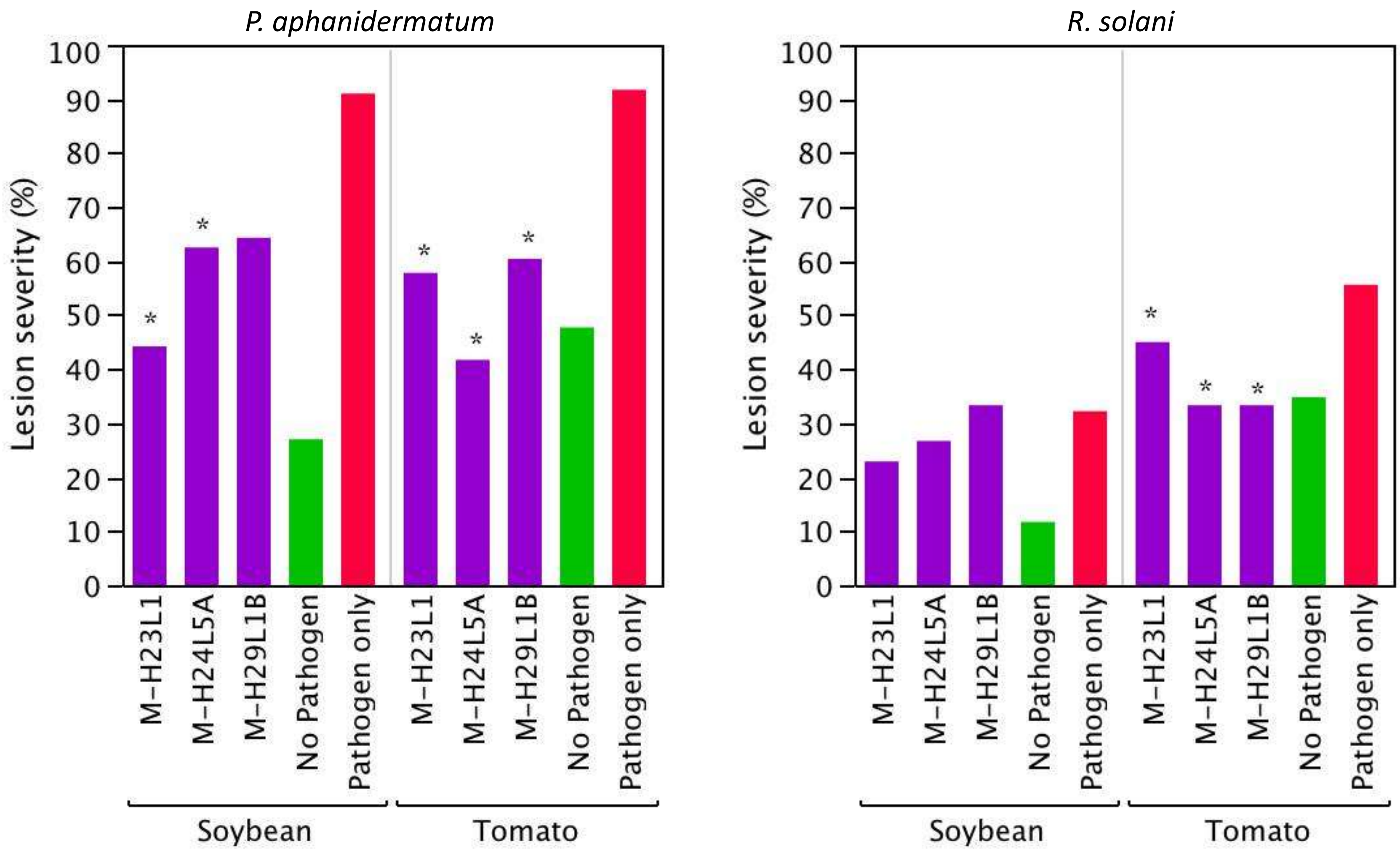




Genera *Incertae* (Burkholderiales)

Can Lead To The Recovery of Novel Actives

…with demonstrated biopesticidal activities



Benitez and McSpadden Gardener 2009 *Appl. Environ. Microbiol.*

The Phytobiome Perspective

- Intensive population monitoring
  - Define when and how plant hosts respond to inputs and stresses
  - Define natural ranges of pathogens, pests and native biocontrol populations which might ameliorate measured efficacy

- Molecular Community Profiling
  - Quantify associations of multiple populations to plant health
    - Identify and recover novel active ingredients
    - Identify “most important” populations
  - Define conditions leading to the emergent property called “plant health”

Phytobiomes Link To Systems Biology

EMERGENT PROPERTIES

"It is thus likely that over the coming years and decades biological sciences will be increasingly focused on the systems properties of cellular and tissue functions...These properties are sometimes referred to as 'emergent' properties since they emerge from the whole and are not properties of individual parts" (72).

"The scientific meaning of emergent, or at least the one I use, assumes that, while
separate parts, its bel
from the nature of it
interact" (19).

SYSTEMS BIOLOGY

"Systems biology...investigates the behavior and relationships of all of the elements in a particular biological system while it is functioning. These data can then be integrated, graphically displayed, and ultimately modeled computationally" (38).

A FRAMEWORK FOR SYSTEMS BIOLOGY

1. Define all of the components of the system.
2. Systematically perturb and monitor components of the system.
3. Reconcile the experimentally observed responses with those predicted by the model.
4. Design and perform new perturbation experiments to distinguish between multiple or competing model hypotheses.

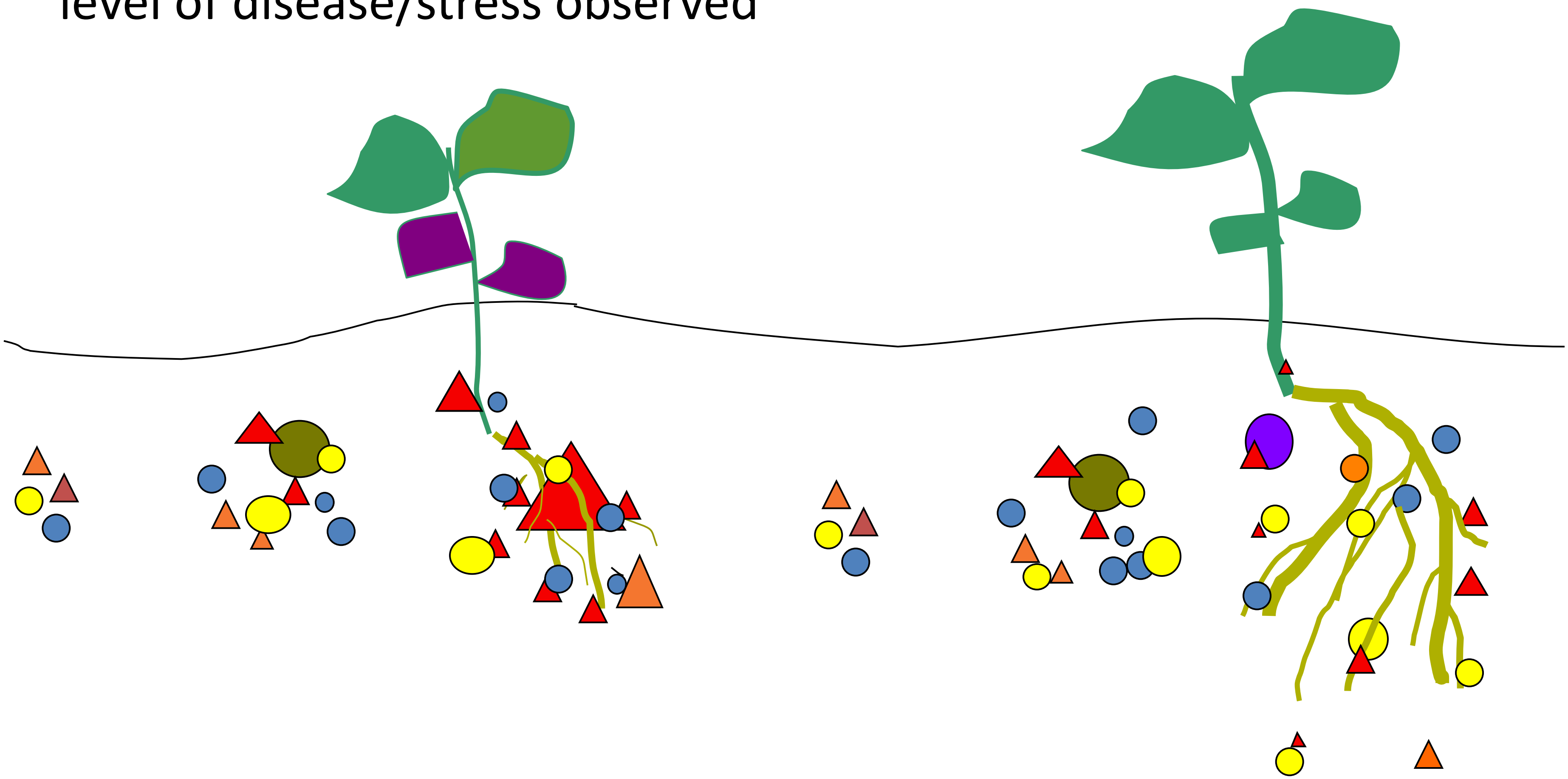
Schneider and Collmer 2010 *Ann Rev Phytopathol*

Consider a General Ecological Model

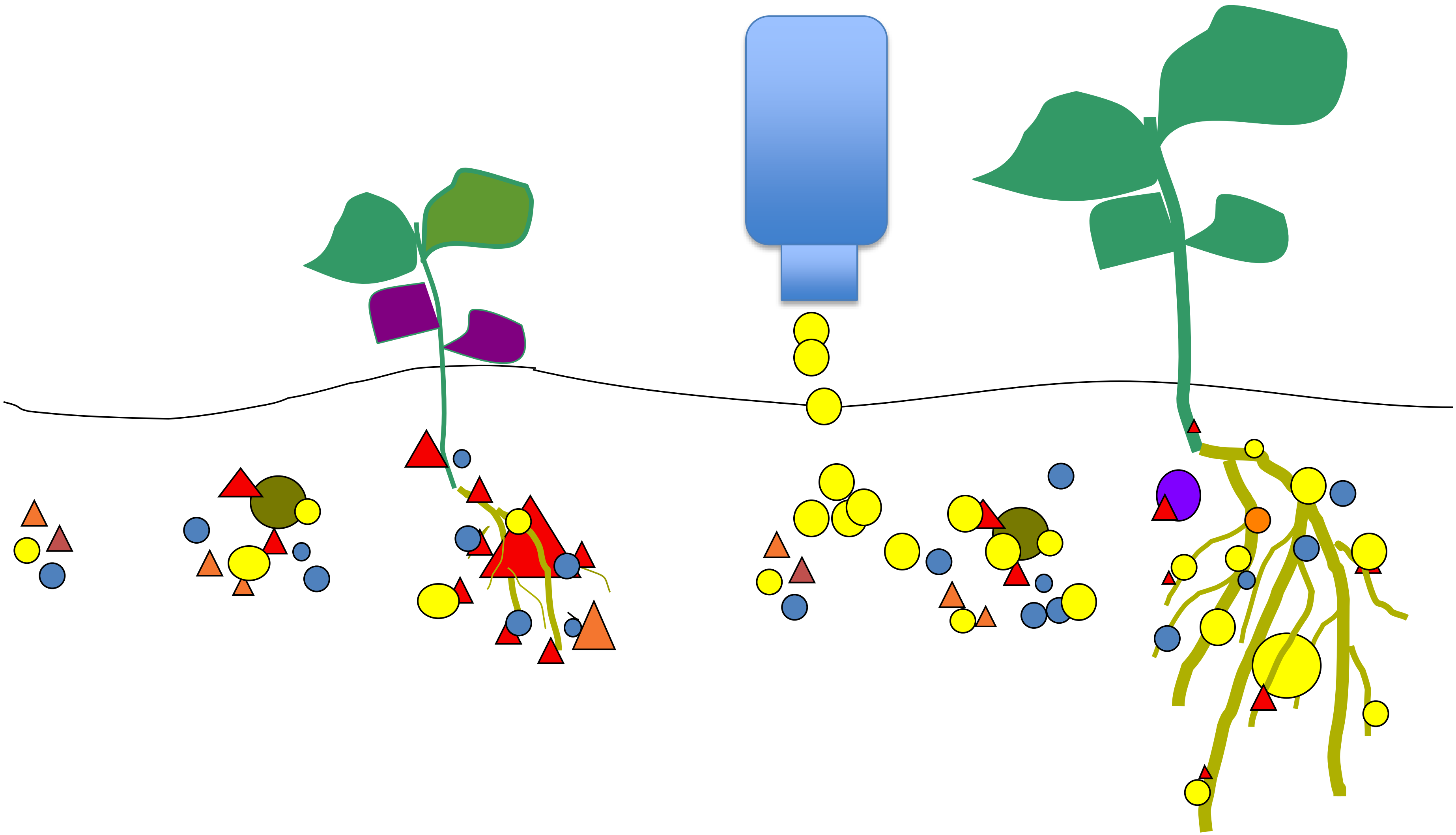
Microbial communities develop around each plant

A mix of pathogens ▲ and beneficials ● determine plant health

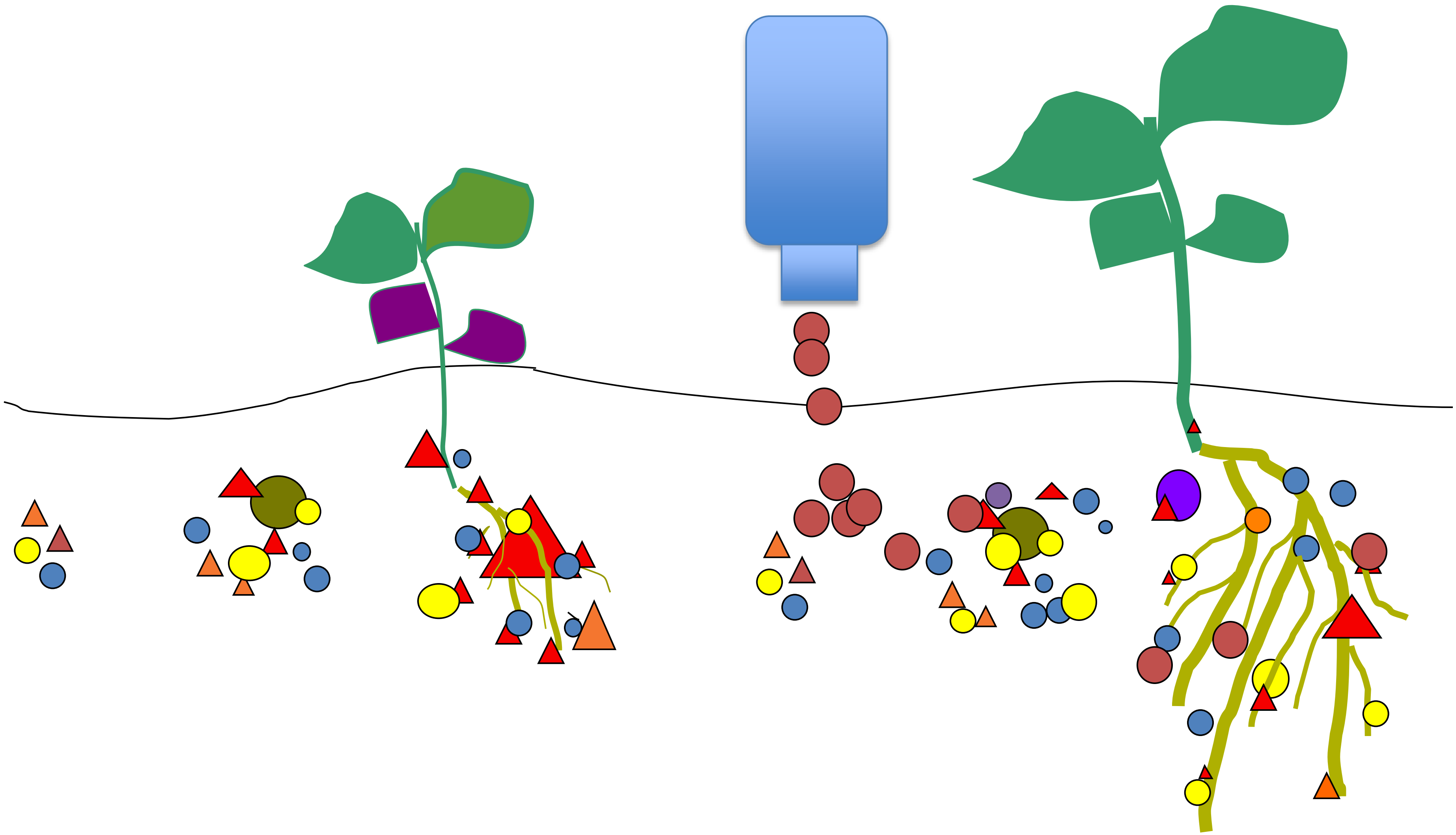
Host, environment, and pathogen/pests interact to determine the level of disease/stress observed



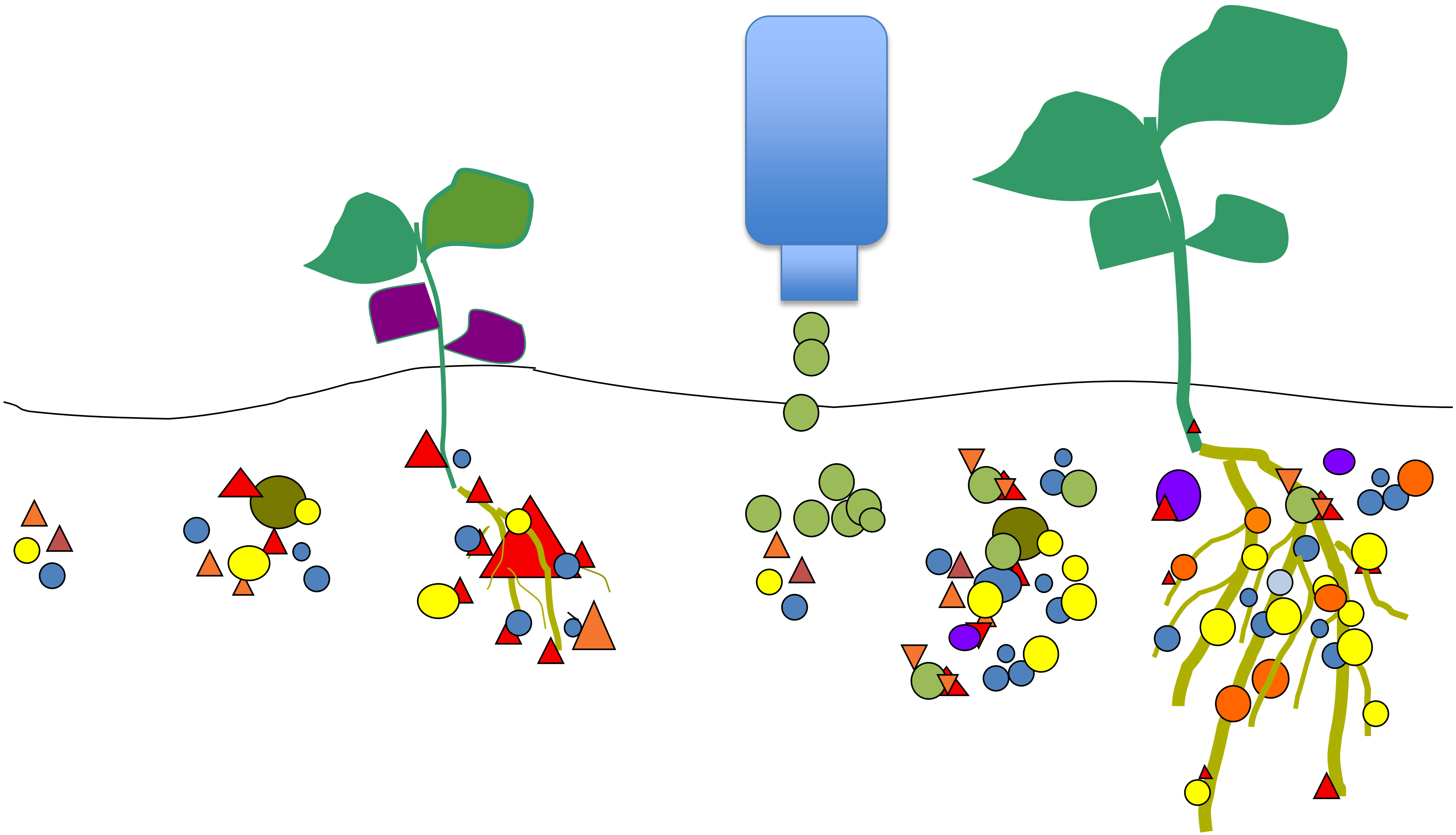
Inoculants may include beneficial microorganisms ● that promote plant growth and health through a variety of mechanisms



Inoculants may include essential mineral nutrients, amino acids, or phytohormones that support root growth and development



Inoculants may include mixtures of compounds that stimulate soil biology; enhancing nutrient cycling, altering WHC, and/or suppressing pathogens and pests to promote plant health



Paradigm Shift

Biocontrol Principles Revised for the 21st Century

- The *dynamic* environmental context (including all biotic and abiotic factors) determines the ecology of the system, the outcome of which will include more or less disease on any given plant
- Plant health status is mediated by *all* biological components (including humans) to varying degrees in the system being studied
- BC is mediated through the interactions of *multiple organisms*, their *diverse multifunctional secretions*, and the *multiple local and systemically integrated responses* of the plant host
- BC is augmented (directly) through inoculation, host manipulation, application of chemicals, and (indirectly) through management of abiotic factors in *every* cropping system

McSpadden Gardener 2010 *APS Mtg*

Paradigm Shift

Some Implications for R&D

- Fundamental studies will utilize more “omics” approaches for characterizing plant health-affecting phenomena under “field relevant” conditions
- Studies will more frequently examine effects of farm practices on all plant-associated microbes associated with changes in plant health status (BC agents an pathogens alike)
- More studies on host-symbiont pairings and multifactor integration will be needed to better manage plant microbial ecology
- Studies will utilize more intensive and extensive sampling regimes and multivariate statistical analyses will become more widely used
- Biocontrol systems and products will use combinations of actives to provide more durable and effective plant disease protection

McSpadden Gardener 2010 *APS Mtg*

Ohio Agricultural Research and Development Center

Plant Microbial Ecology and Biopesticide Development Laboratory

- Applied research and development
  - Quantify the effects of microbial inoculants on plants (in terms of growth, health status, stress tolerance, harvestable yield, *etc*.)
  - Develop new methods and protocols for monitoring levels of microbial biopesticides in the environment
  - Formulate and field test new strains of microbial biopesticides and biofertilizers for stability and efficacy