Methylotrophs for Sustainable Agriculture
PAG XXV – Exploring Phytobiomes Workshop

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NewLeaf SYMBIOTICS

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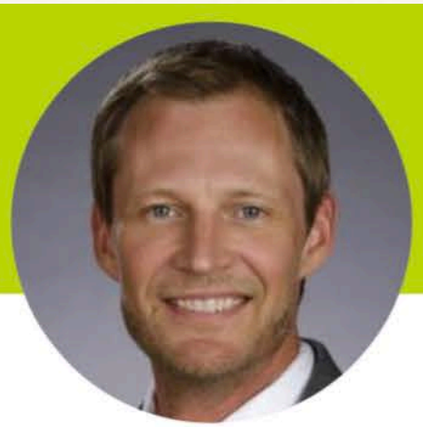

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Microbials and the Future of Agriculture

- *Challenge:* adoption of sustainable agricultural technologies to feed 9 BB people
- New GM crop costs 10 years and $150 MM
- New Ag chemical costs 12 years and $250 MM
- Microbial solutions have accelerated path-to-market
- Mainstream Agriculture embraces Microbials as major disruptive technology and growth opportunity

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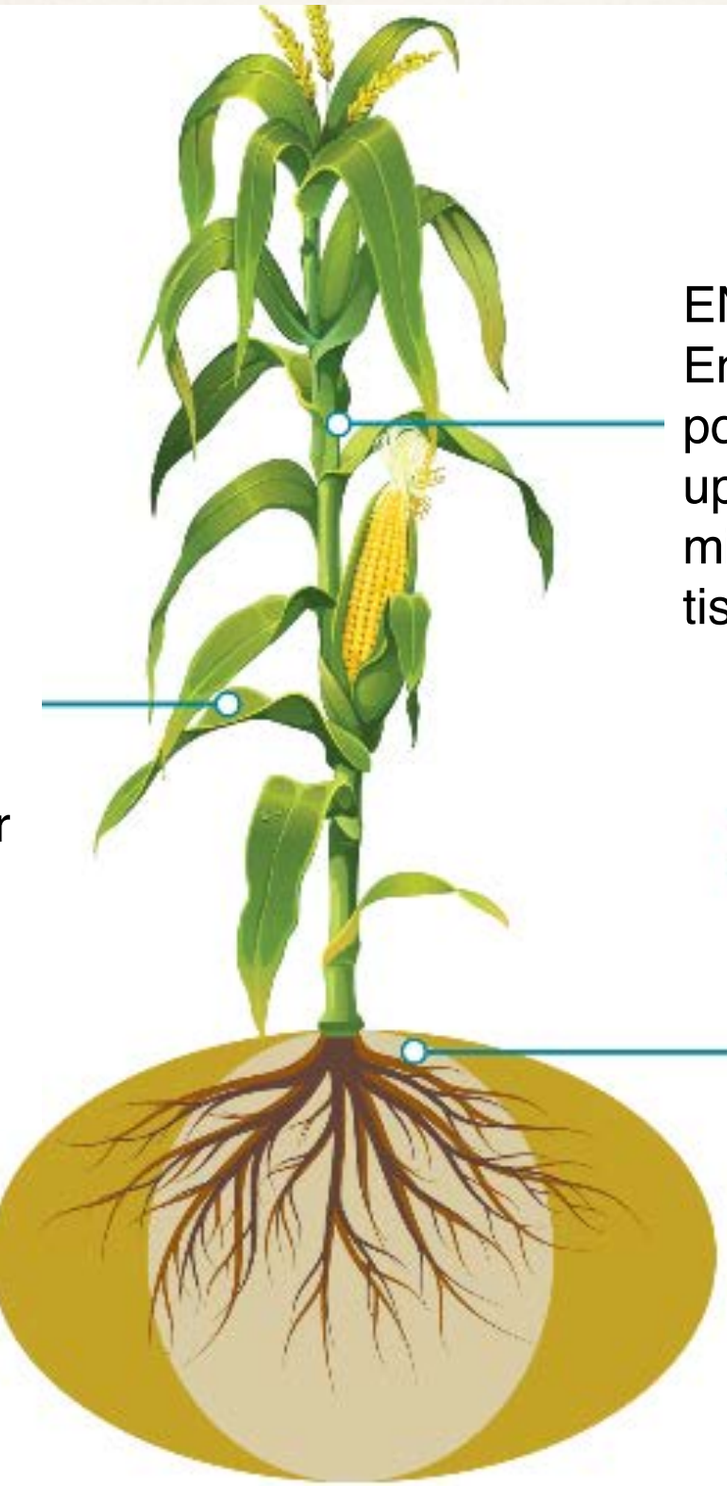
Microbiome – "Second Plant Genome"

PHYLLOSPHERE
A plant's leaves and stems can have up to 10 million microbes per square centimeter

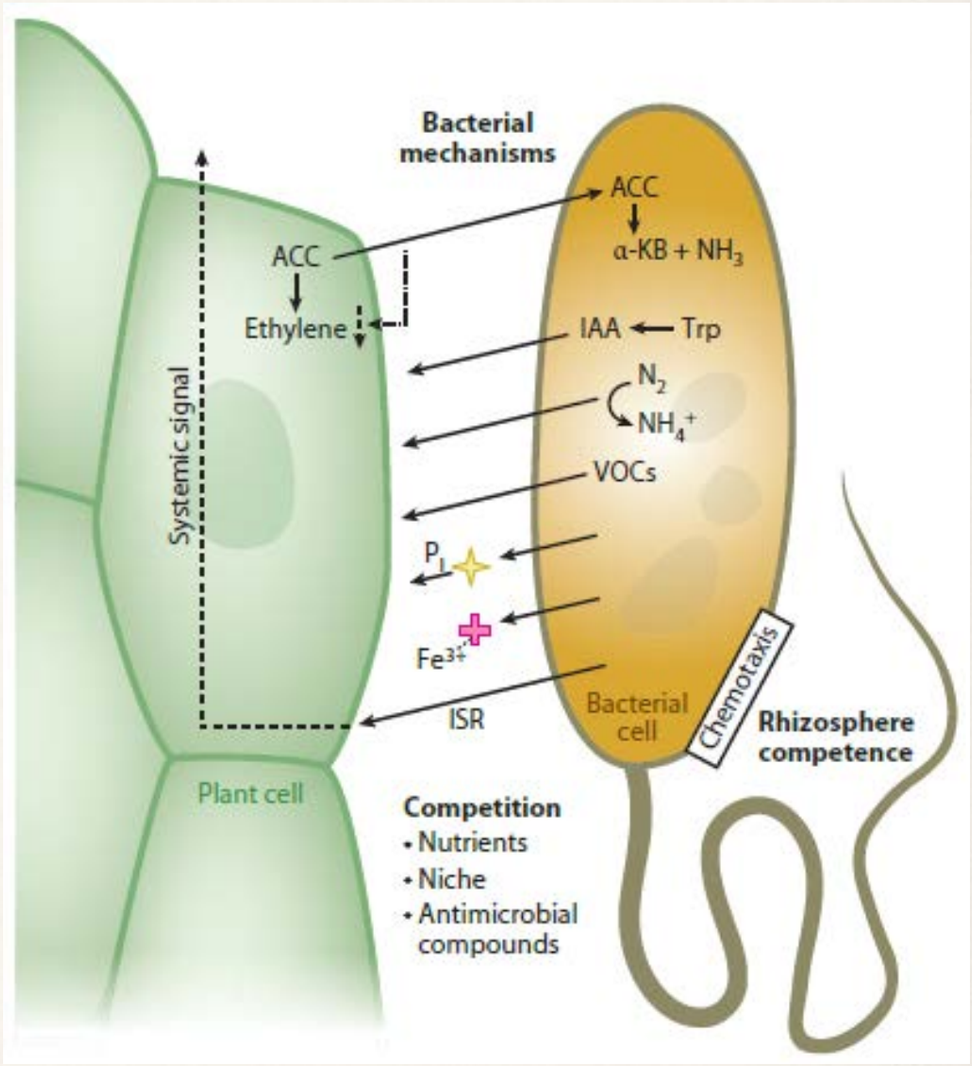


ENDOSPHERE
Endophytic populations can reach up to 10 million microbes per gram of tissue

RHIZOSPHERE
1 gram = up to 10 billion microbes and up to 30,000 different species

Microbial Plant Enhancement "Traits"

- Phytohormones
  - IAA production
  - ACC deaminase
  - Acetoin, 2,3 butanediol etc.
- Biotic stress mitigation
  - Induced Systemic Resistance
  - Biopesticidal compounds etc.
- Abiotic stress mitigation
  - Drought (WUE, increased rooting, ABA), temperature, salinity
  - Reduction of ROS
- Nutrient acquisition
  - Phosphate solubilization
  - Nitrogen fixation
  - Iron scavenging etc.



Bulgarelli *et al.* (2013)

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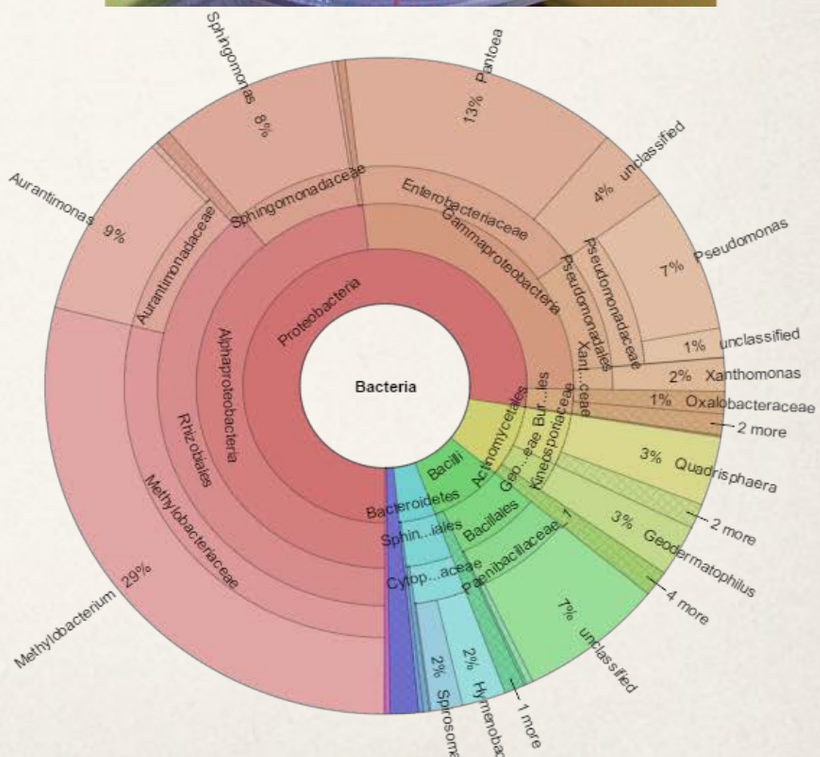
NewLeaf Symbiotics Strategy

- Methylotroph-based Microbial products as drop-in solutions for sustainable agriculture

- Methylotrophs are ubiquitous colonizers of plants (phylloplane, rhizosphere, endosphere)

- Rich in Plant Enhancement "Traits"

- Discover and Develop the best fit strain(s) to each product concept (crop, BioYield, BioControl, application method)



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NewLeaf Is Mining a Rich Vein

- ~1,500 Methylotrophic strains from roots, leaves and endosphere of wild crops (~7,000 strain by end 2017)
- All genomes sequenced, assembled and annotated
- Plant enhancement "trait" discovery rate is very high
- Very "productizable" for non-spore formers

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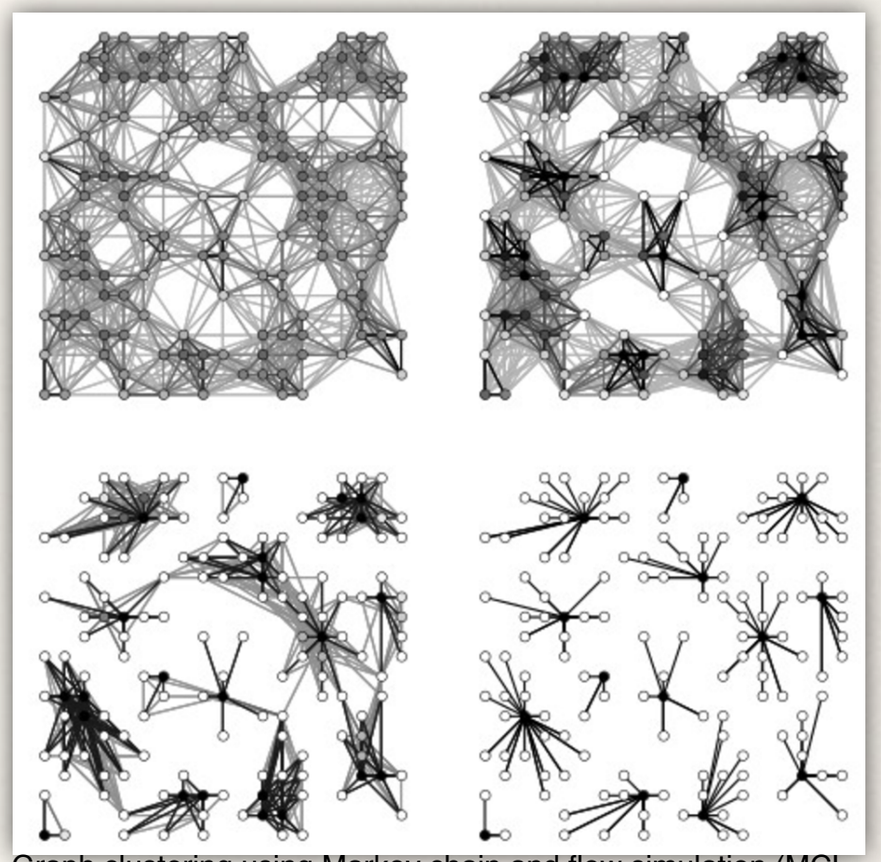
Methylotroph Pan-genome Analysis

- Typical Methylotroph genome
  - ~ 5,200 genes per genome
  - 54% genes with assigned function
  - 46% genes no assigned function (70% of gene clusters no assigned function)

- Align all protein sequences from all genomes against themselves

- Cluster genes around protein homology
  - Core genes: >= in 95% genomes
  - Shell genes: in multiple genomes
  - Cloud genes: in only few or one genome



Graph clustering using Markov chain and flow simulation (MCL).
Image Credit: Stijn van Dongen, Graph Clustering by Flow Simulation. PhD thesis, University of Utrecht, May 2000.

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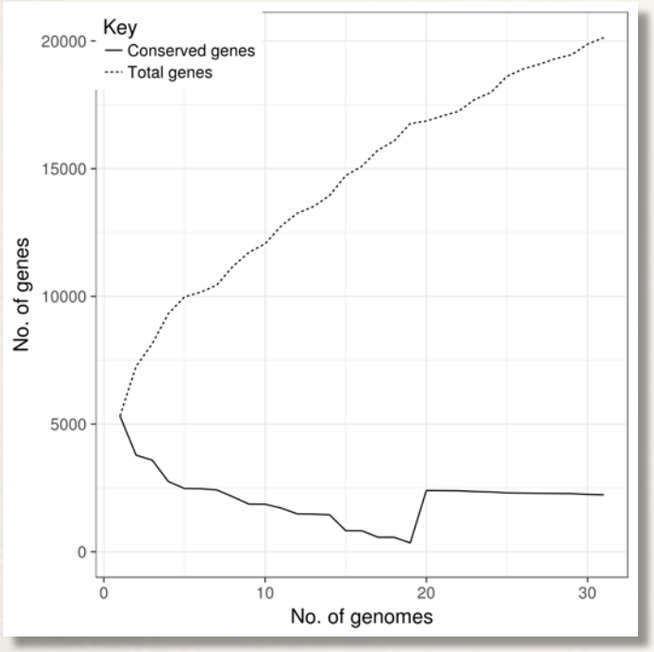
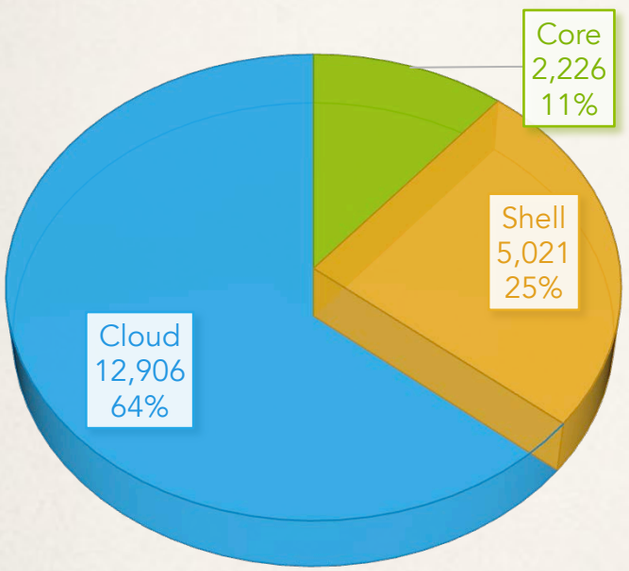
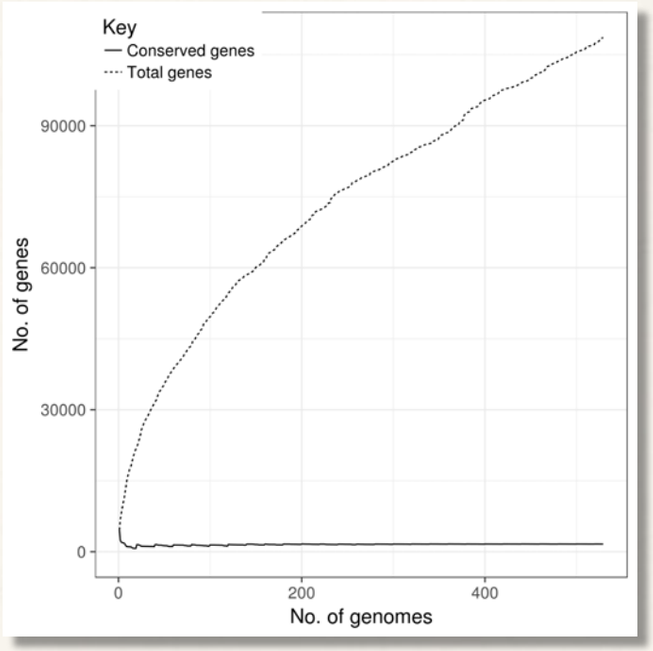
Genes Over Genomes

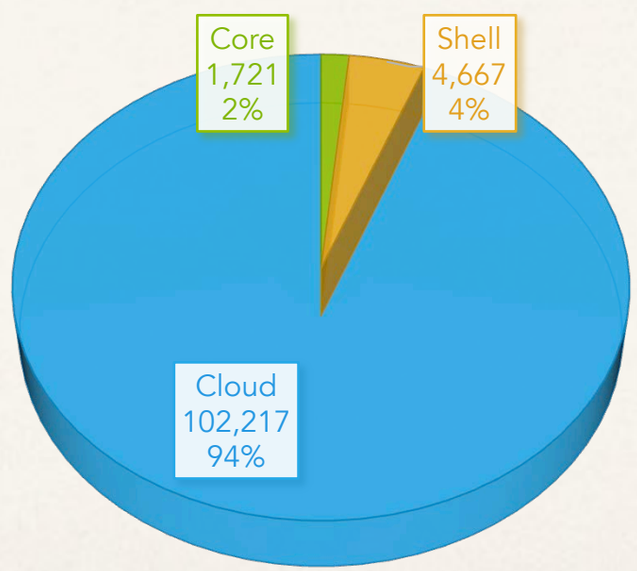


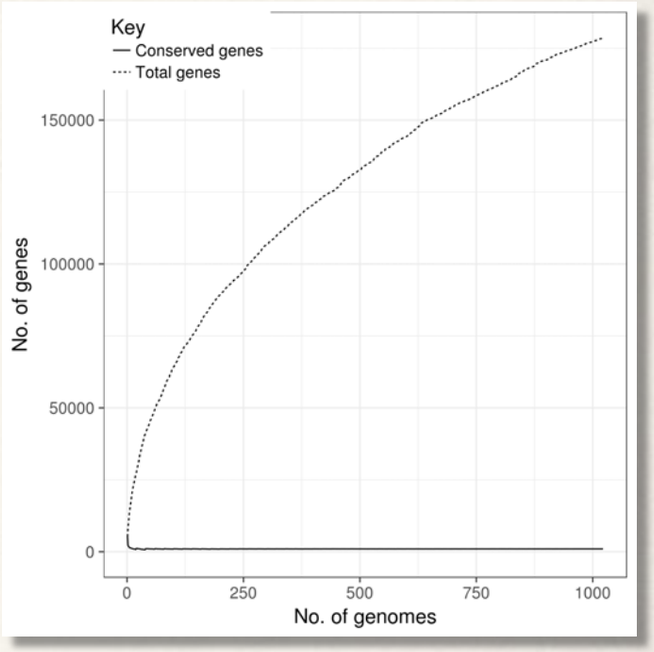
M. populi, 30 genomes



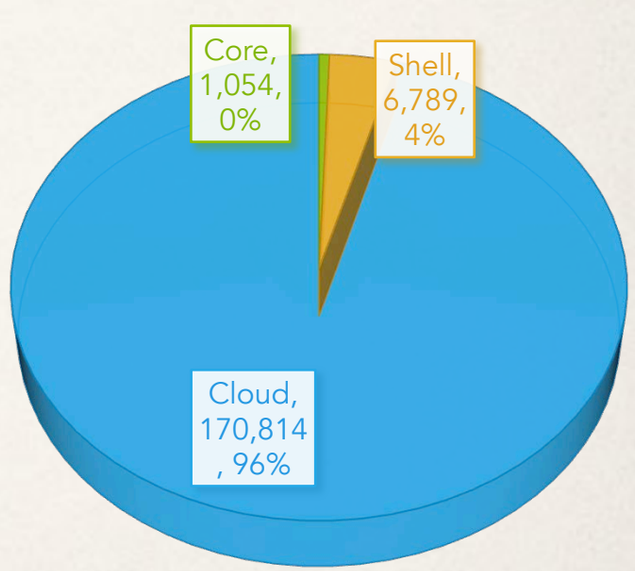


M. extorquens, 500 genomes



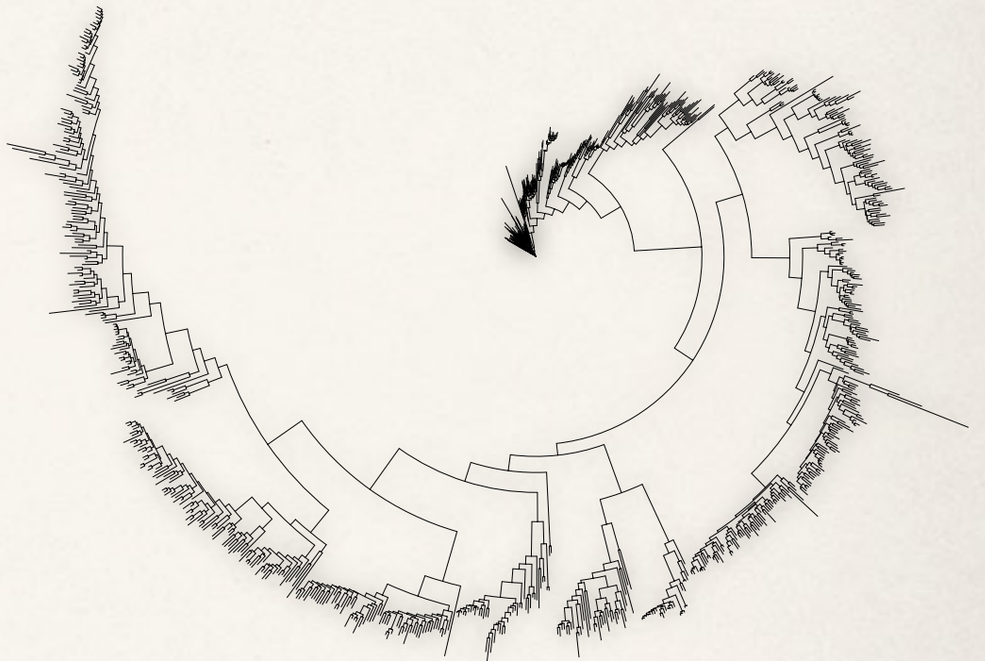


Methylobacterium, 1000 genomes

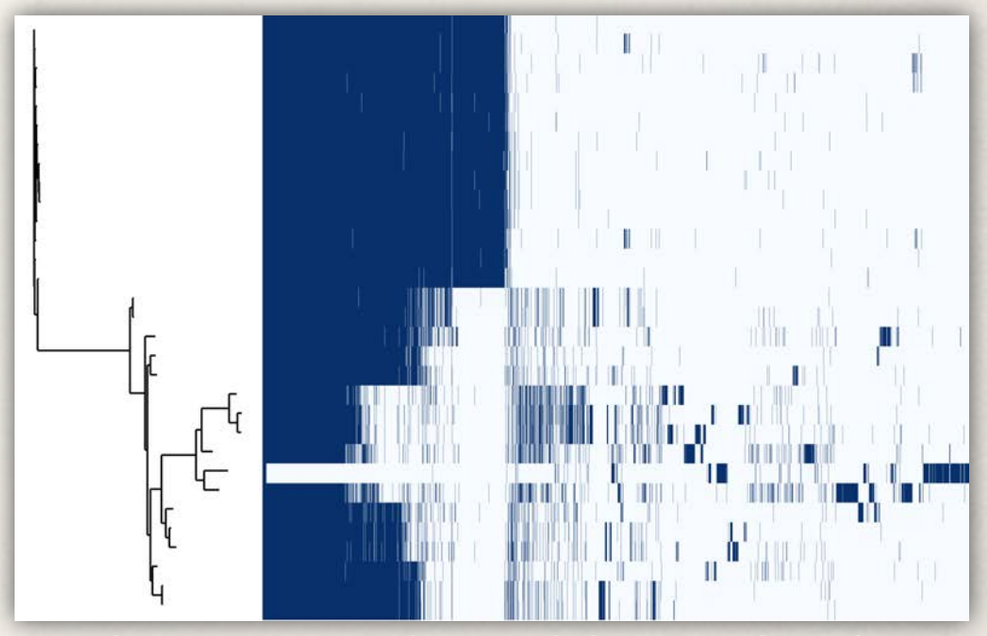


Gene Presence Phylogeny

- Pan-genome enabled analysis
- Identify uniquely all genes over all genomes
- Use genes presence/absence to infer distance
- Protein sequences cluster agnostically to function
- Sufficient phenotype data allows genes/traits linkage identification



Methylobacterium, gene presence/absence phylogeny



M. populi, gene presence/absence phylogeny and visualization

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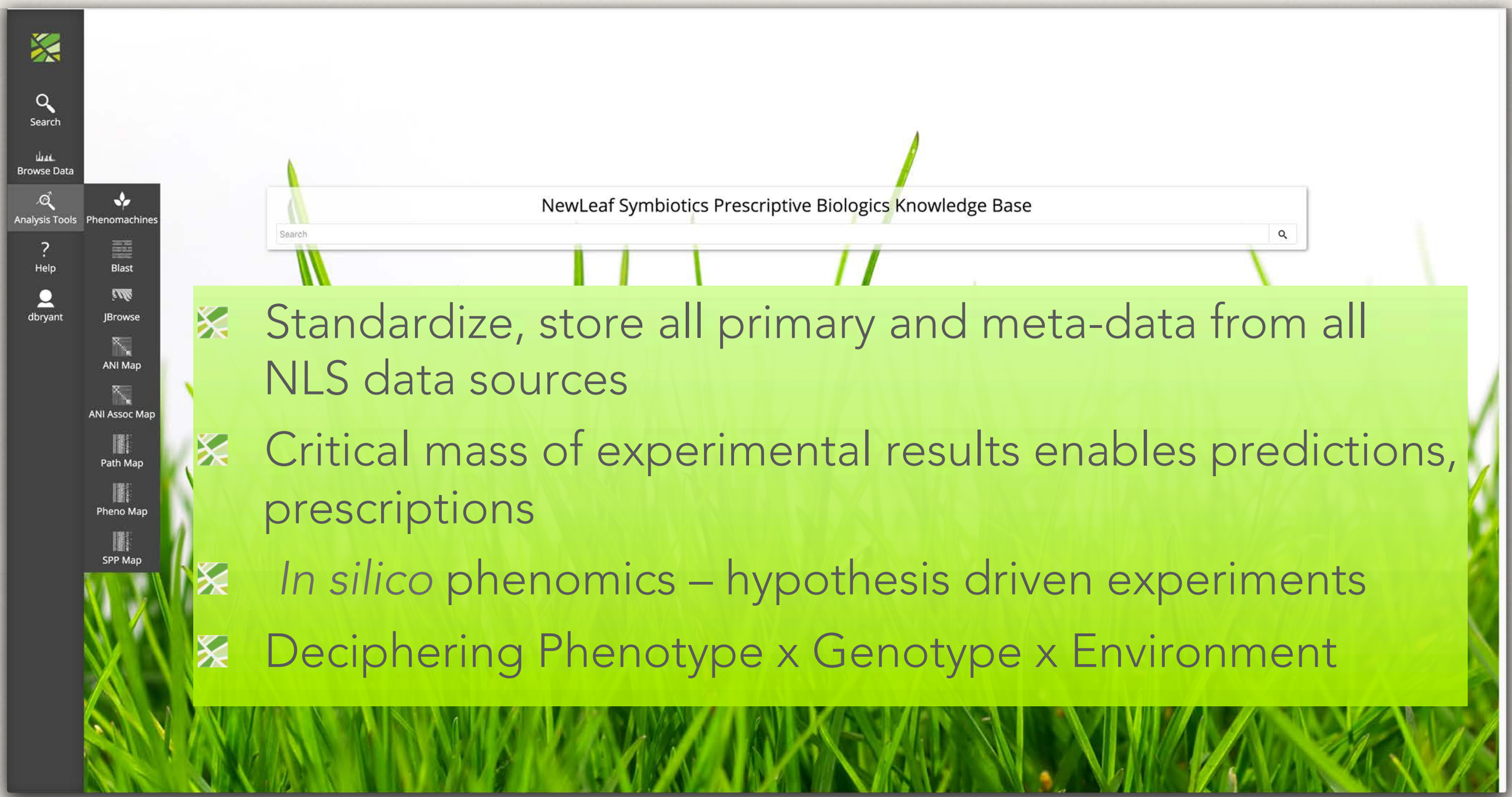
Prescriptive Biologics Knowledge Base™



- Standardize, store all primary and meta-data from all NLS data sources
- Critical mass of experimental results enables predictions, prescriptions
- *In silico* phenomics – hypothesis driven experiments
- Deciphering Phenotype x Genotype x Environment

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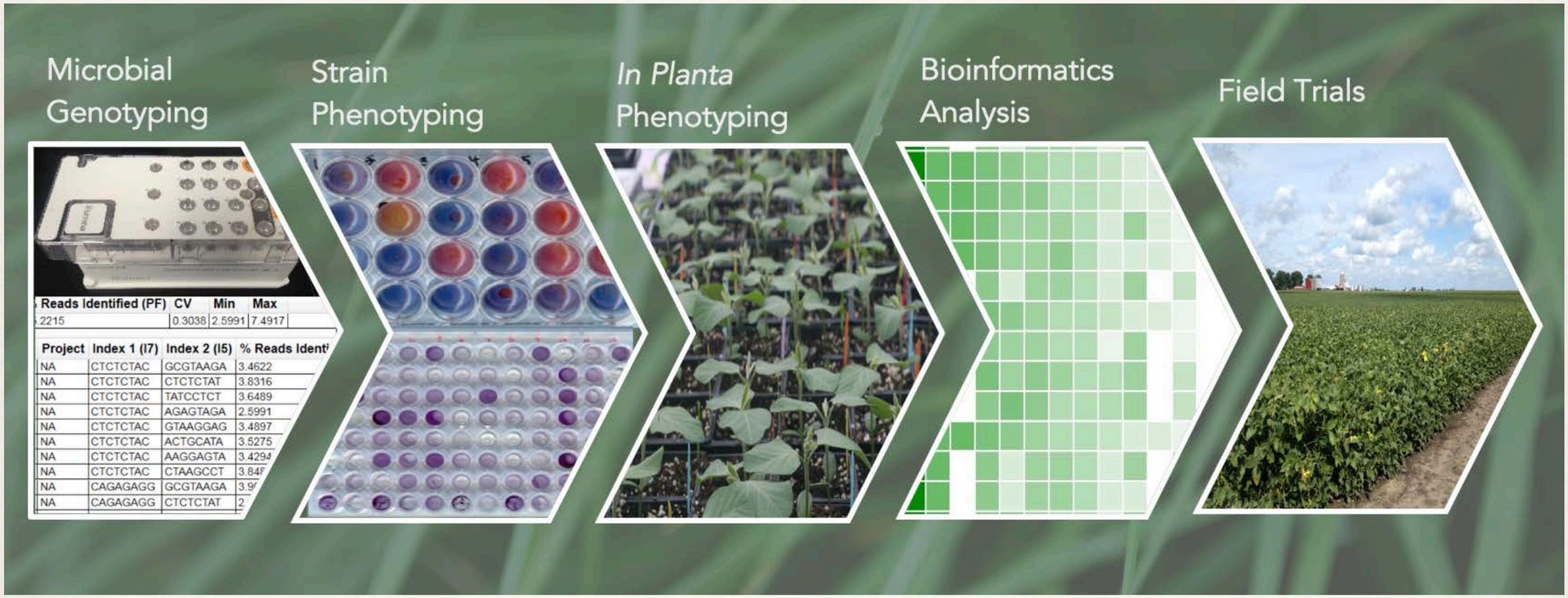
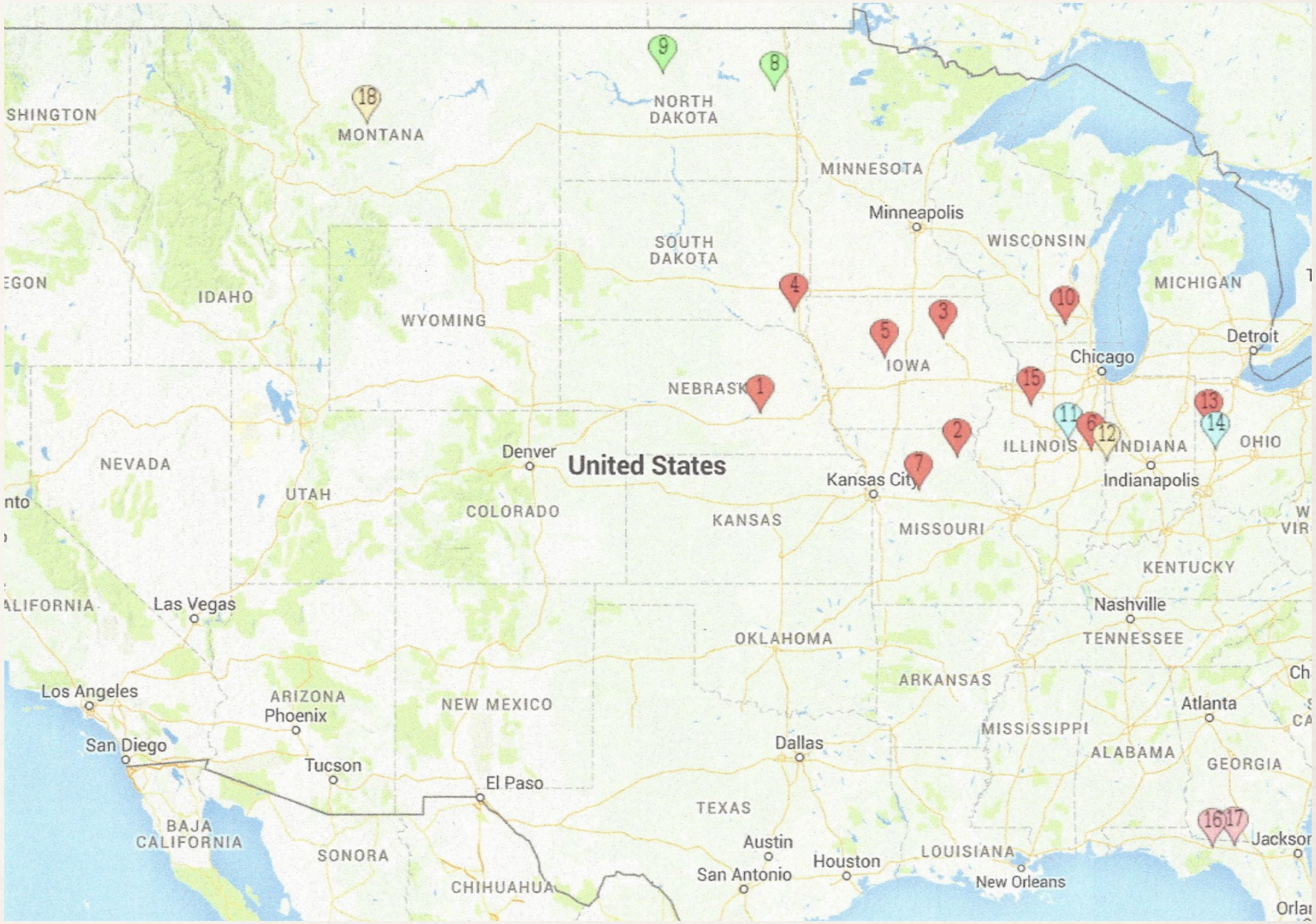
NewLeaf R&D Workflow



US Field Trial Locations

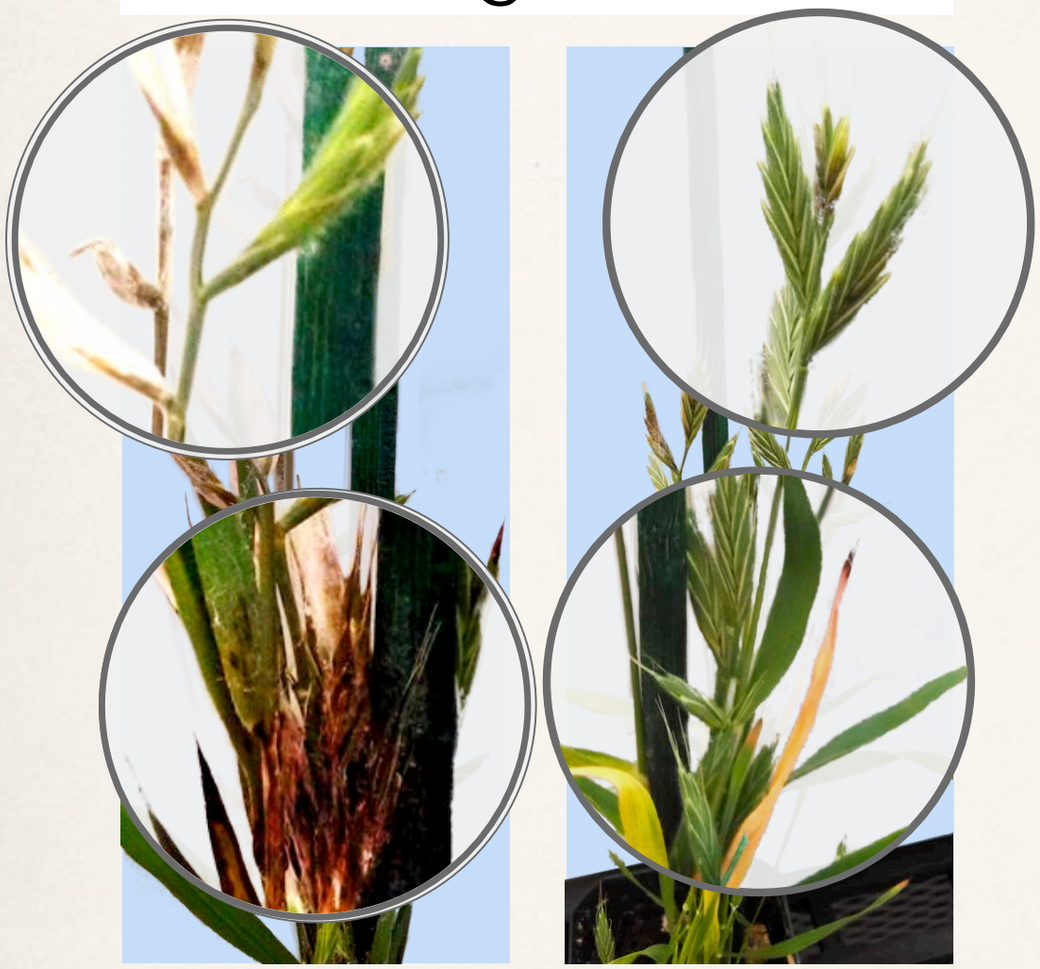
- Corn & soya
- W-wheat, corn & soya
- W-wheat
- S-wheat & soya
- Peanut



Colonization of Plant from Seed

Suppression of Fungal Pathogens

Head Blight - Wheat



Untreated check

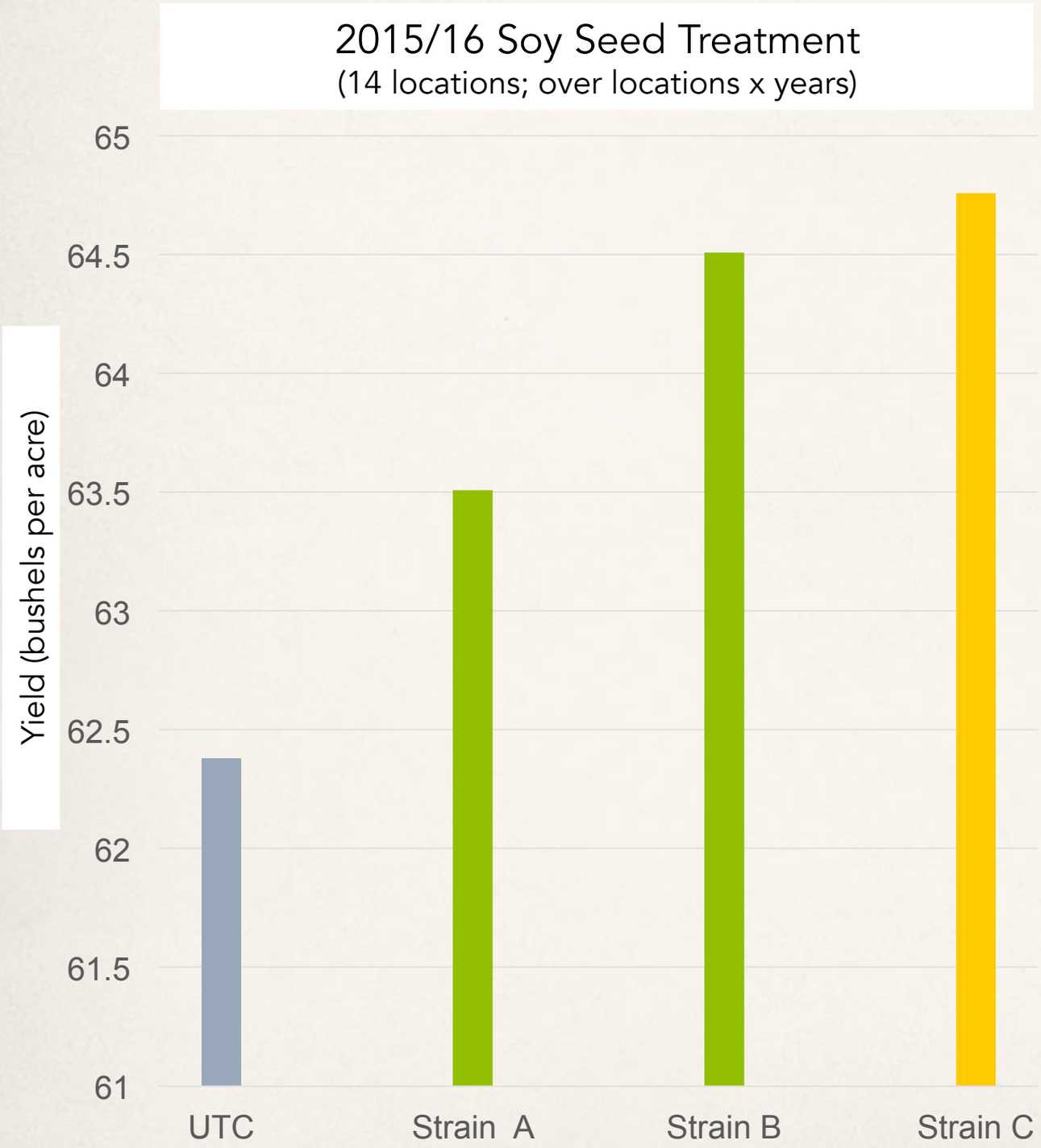
Microbial treatment

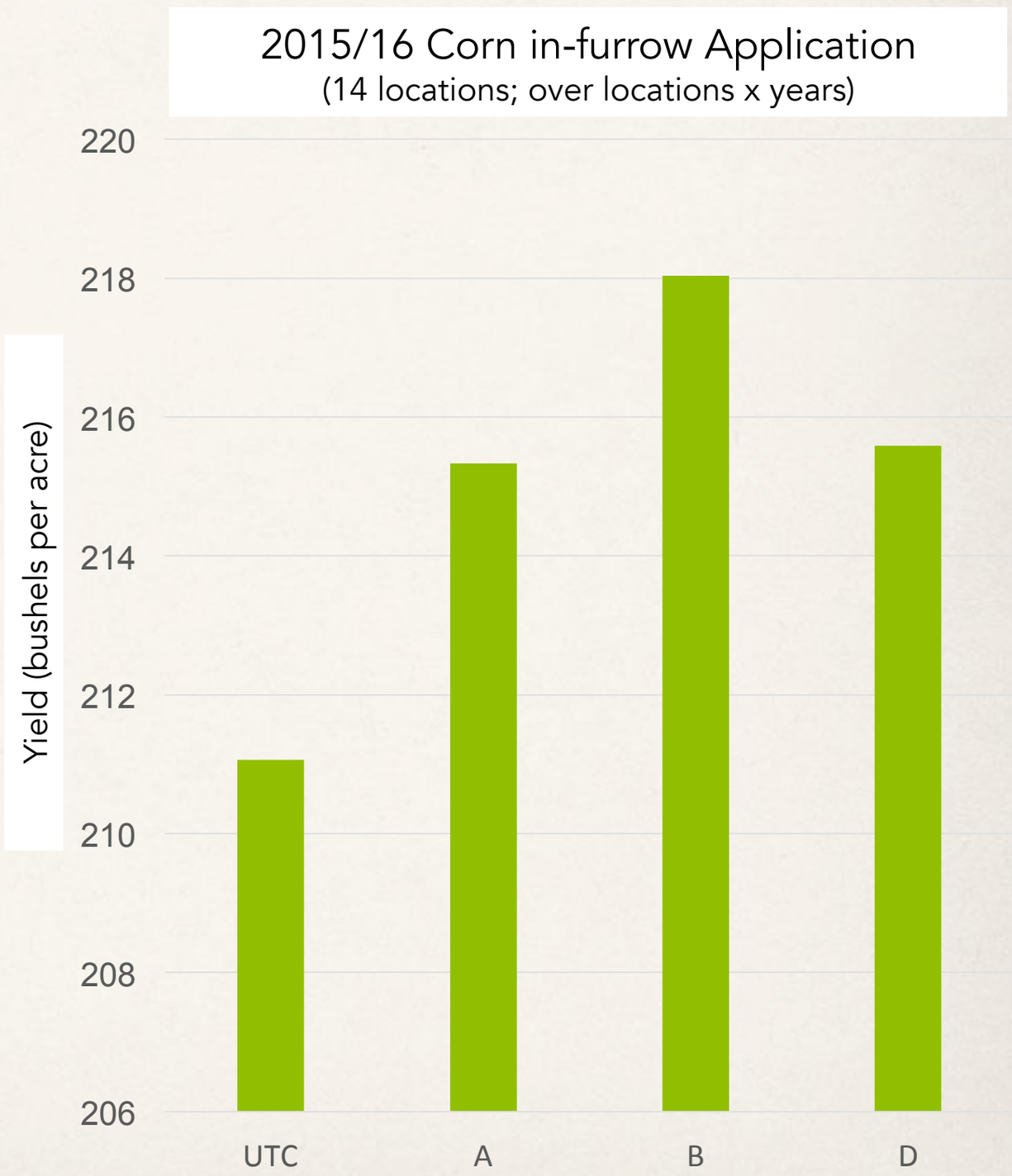
Grey Leaf Spot - Corn

Untreated check

Microbial treatment

Increase of Yield Over Locations x Years

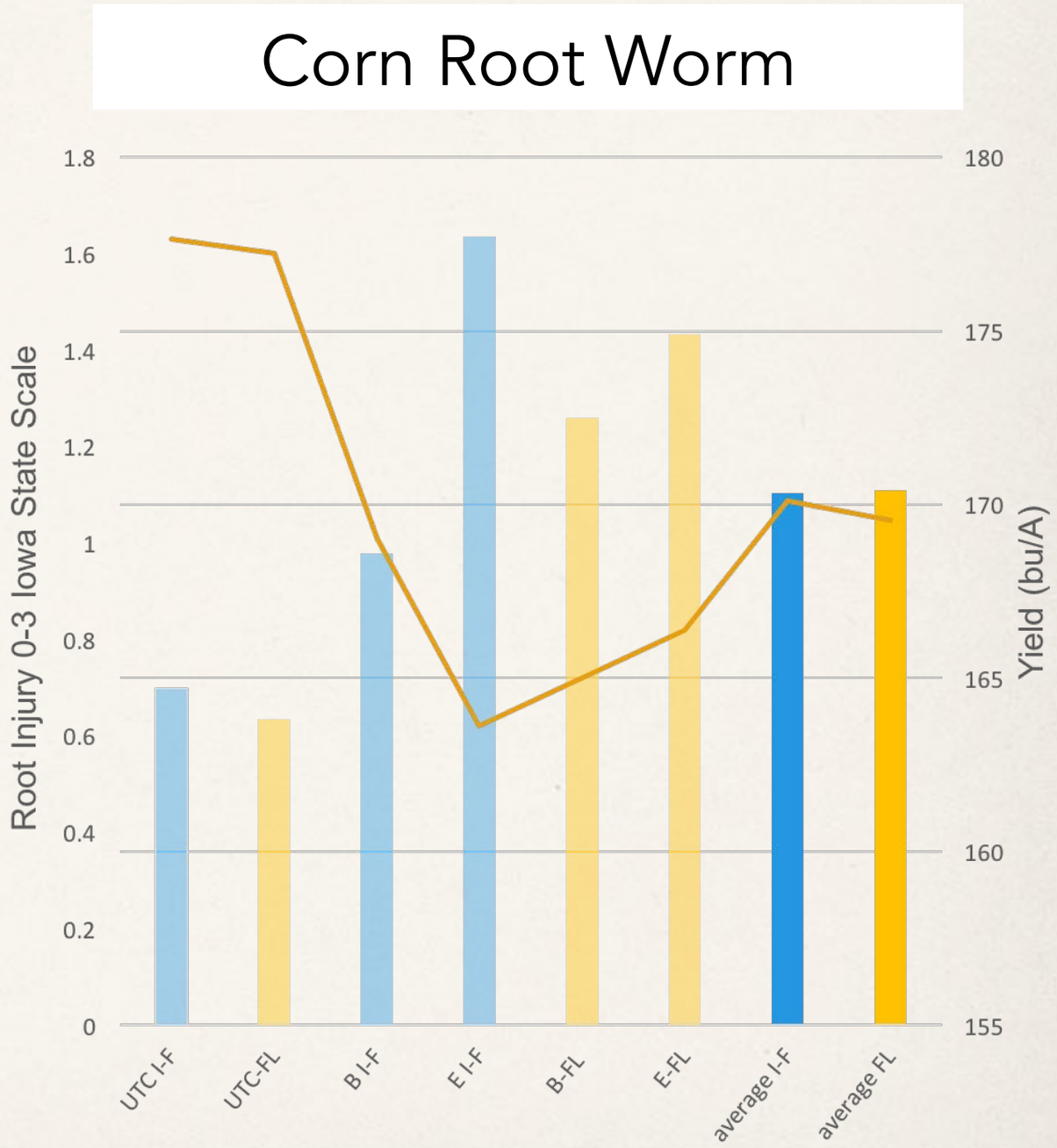




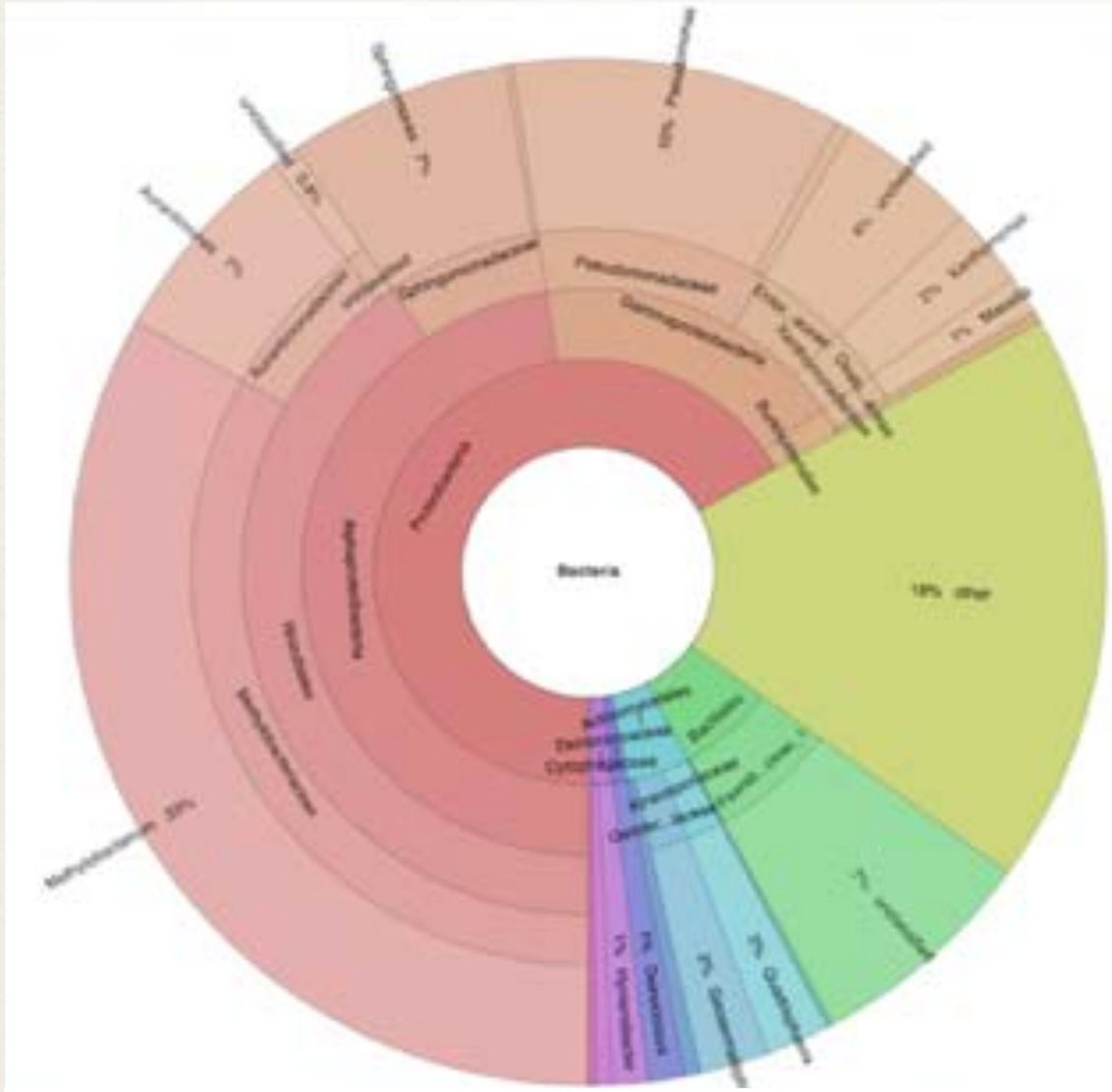
Mitigation of Agricultural Pests

- Lead CRW biocontrol strain delivered:
  - ~ 13 bushel per acre advantage across two years
  - ~60% reduction in root damage across two years
  - Comparable to in-furrow chemical insecticide (pyrethroid)

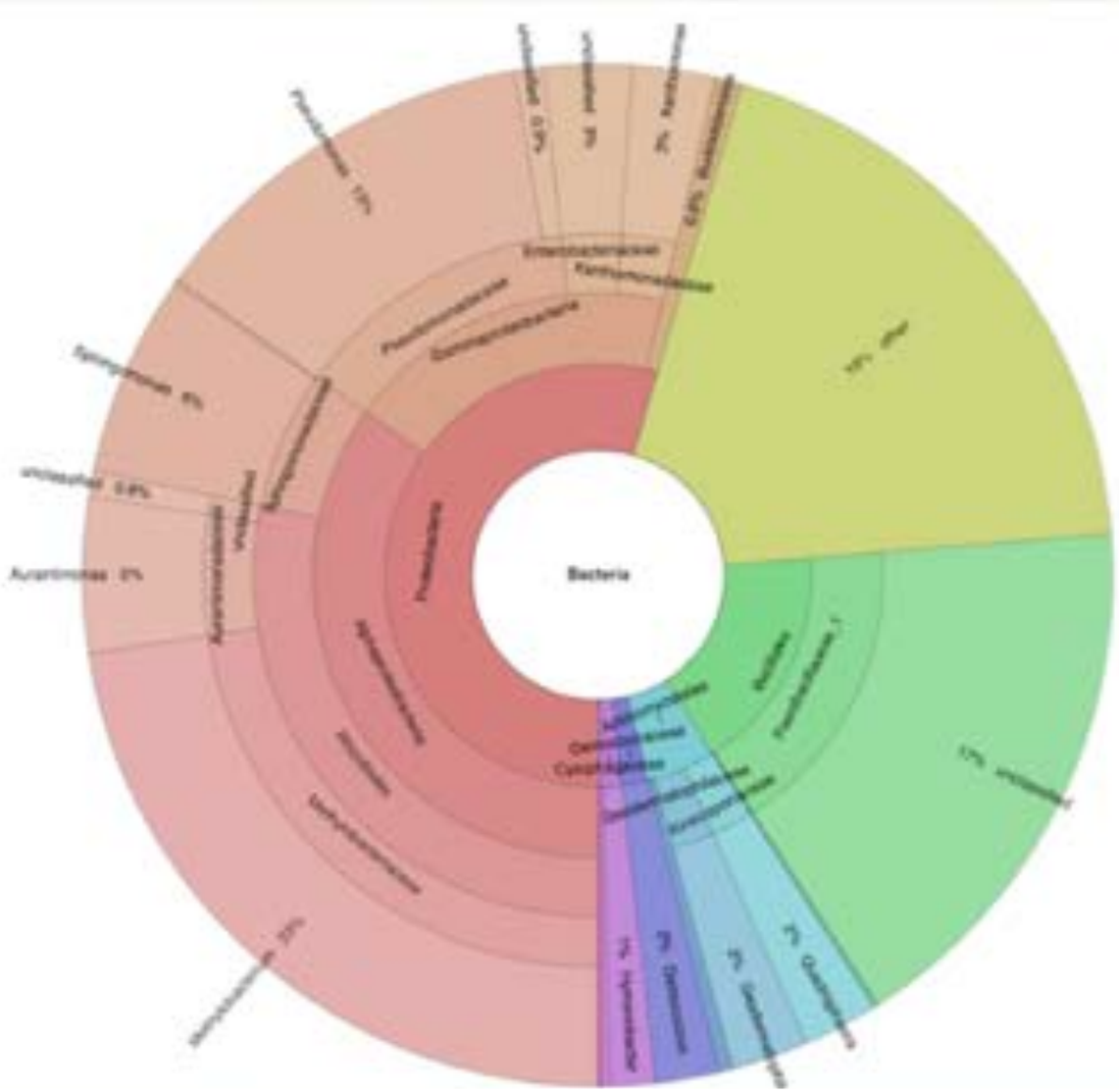
- Lead strain offer an Integrated Pest Management option



Modification of Microbiome



Untreated
check



Microbial treatment
(significant yield advantage)

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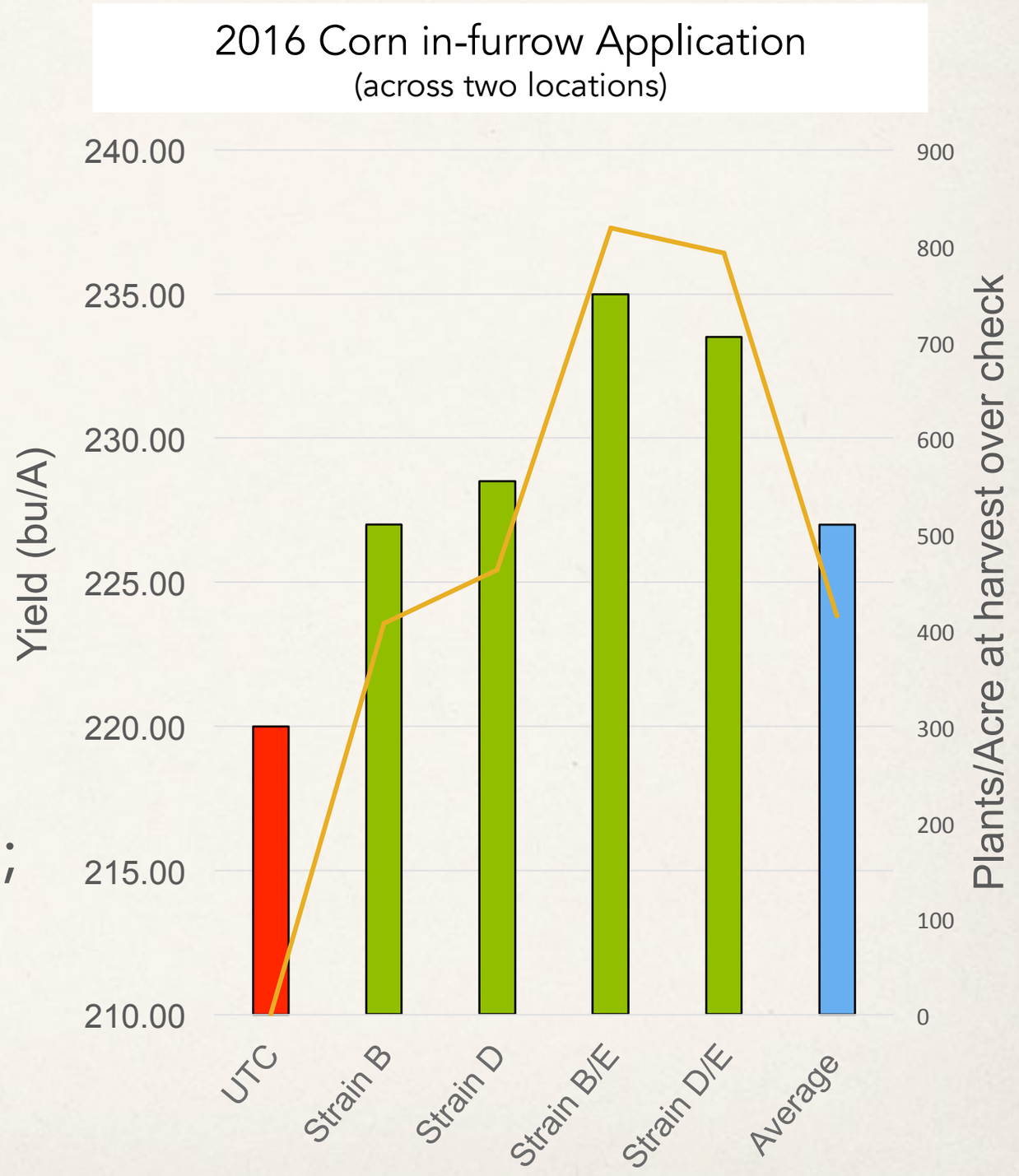
Combination of Lead Strains

- Lead strains provide over seven bushel advantage to the check
- Combination of lead strains with strain E provide additional over seven bushel advantage
- Stand retention delivers yield; resistance to lodging delivers “harvestable” yield at high planting rates



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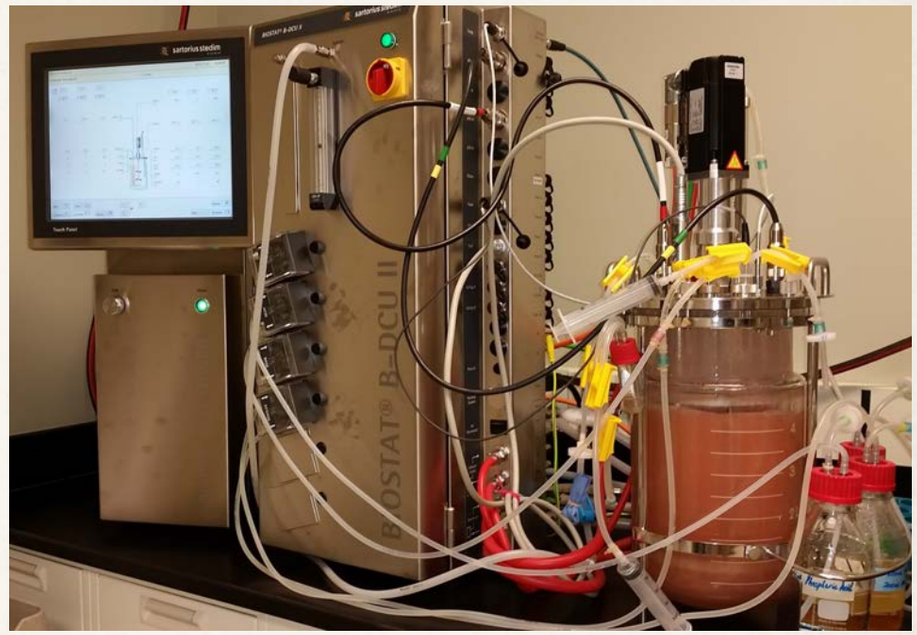
Production and Formulation

- Production patent granted in 2015 provides a unique position (US Patent 9181541)



- Scaling from pilot scale to 100,000L production in 2017

- Formulations are drop-in solutions with current agronomic inputs (traits, chemistries, fertilizers, practices)

- *First sales revenue in US markets in 2017*

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Acknowledgements

NewLeaf Symbiotics

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Private Equity Investments

ROCKPORT CAPITAL

BIO RESEARCH & DEVELOPMENT GROWTH
BRDGPark
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