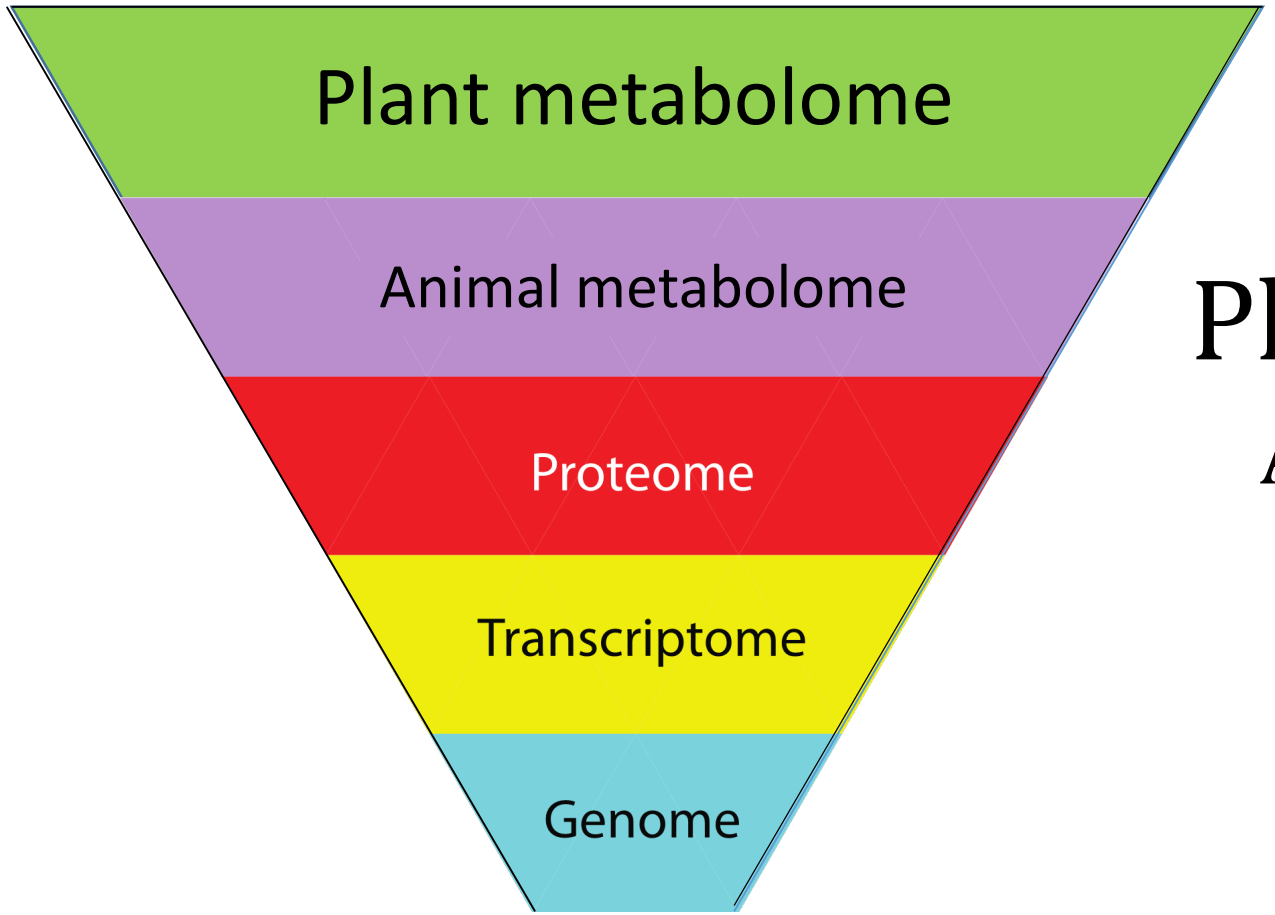
Emerging view of omics complexity



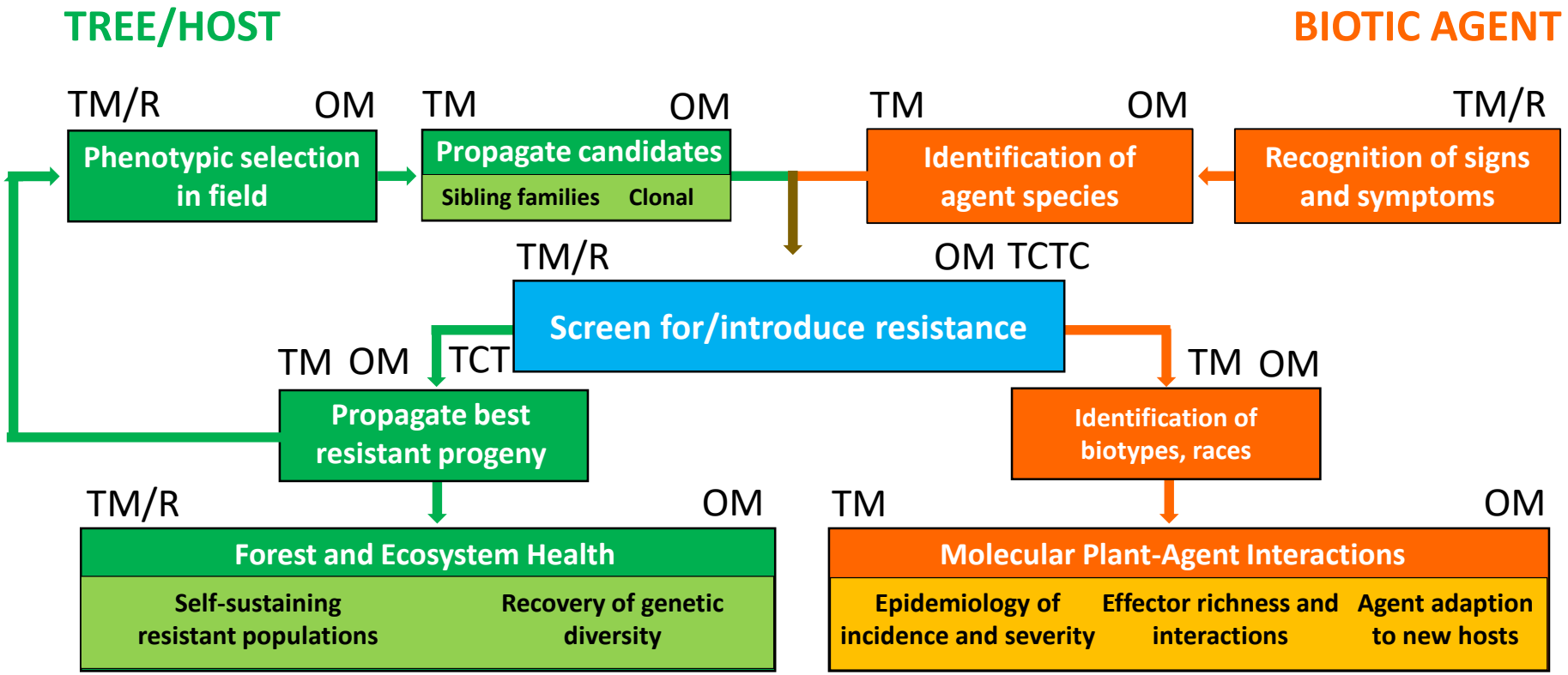
Plant Metabolomics

An undiscovered country

or

Why a metabolomics approach could make sense for TACF

Integrated workflow for breeding for biotic agent resistance

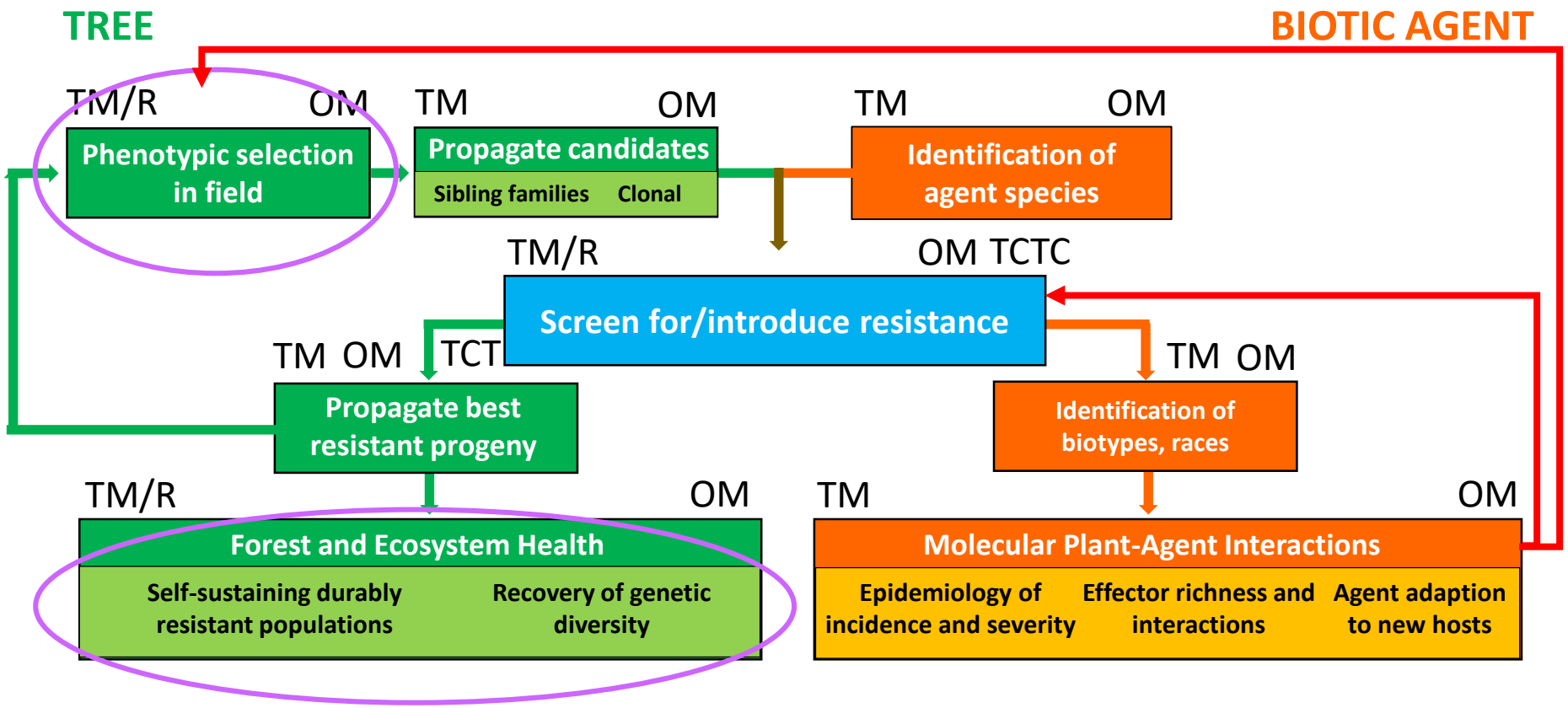


TM = Traditional methods R = Robotics OM = Omics TCTC = Tissue culture, transformation, CRISPR

Original concept for figure from

Keriö, S., H. A. Daniels, M. Gómez-Gallego, J. F. Tabima, R. R. Lenz, K. L. Søndreli, N. J. Grünwald, N. Williams, R. McDougal and J. M. LeBoldus (2019). "From genomes to forest management – tackling invasive *Phytophthora* species in the era of genomics." Canadian Journal of Plant Pathology: 1-29.

Integrated workflow for breeding for biotic agent resistance

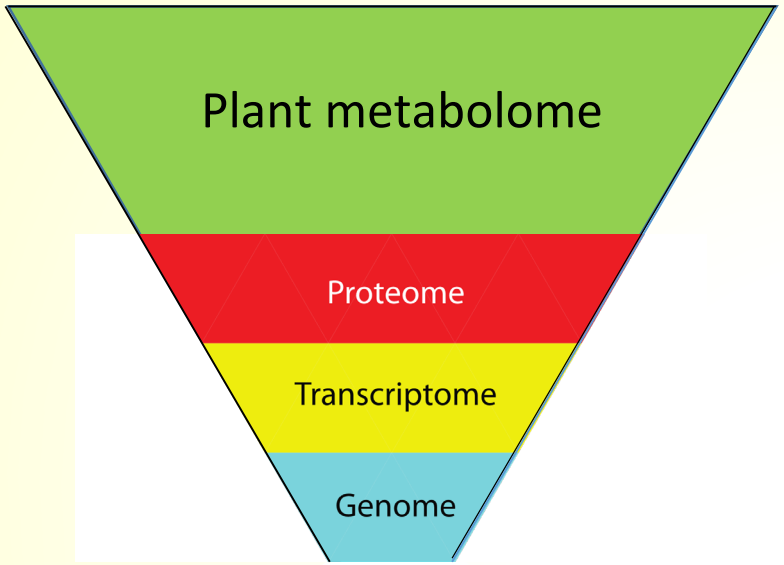


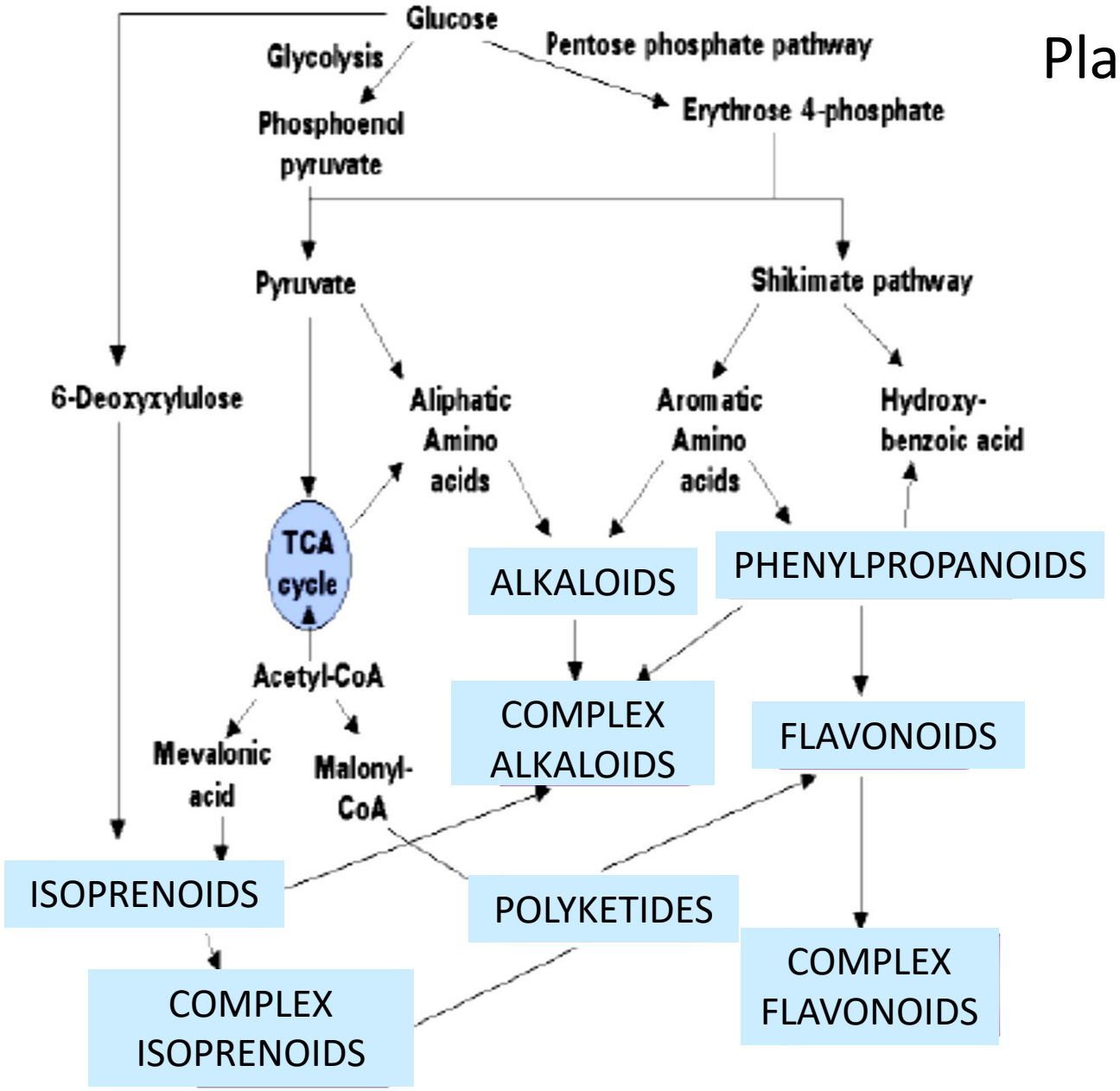
What part of this workflow is the primary goal of the TACF ?

Where is Omics best applied?

The most cost-effective Omics tools

- **Phenomics**
- Omics as near as possible to direct measure
  - **Metabolomics**

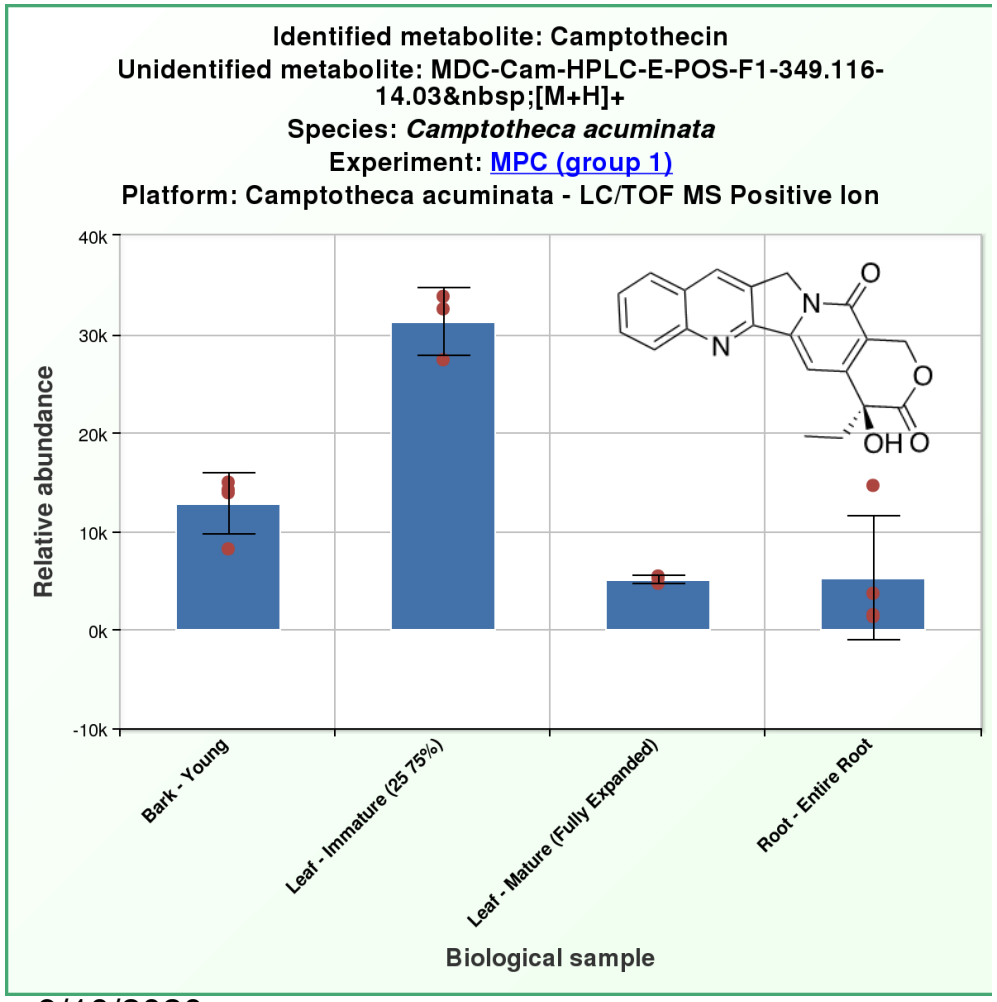




Plant secondary metabolites

- Uses
  - Defense
  - Signaling
- Target specificity
  - Broad to very narrow
- Host intrinsic genetic capacity
  - Extreme variation
    - Species
    - Individuals within species
    - Tissue types within individuals
    - Developmental state within tissue types
- Environment *strongly* influences realized host capacity

In which *Camptotheca acuminata* tissues is camptothecin found?



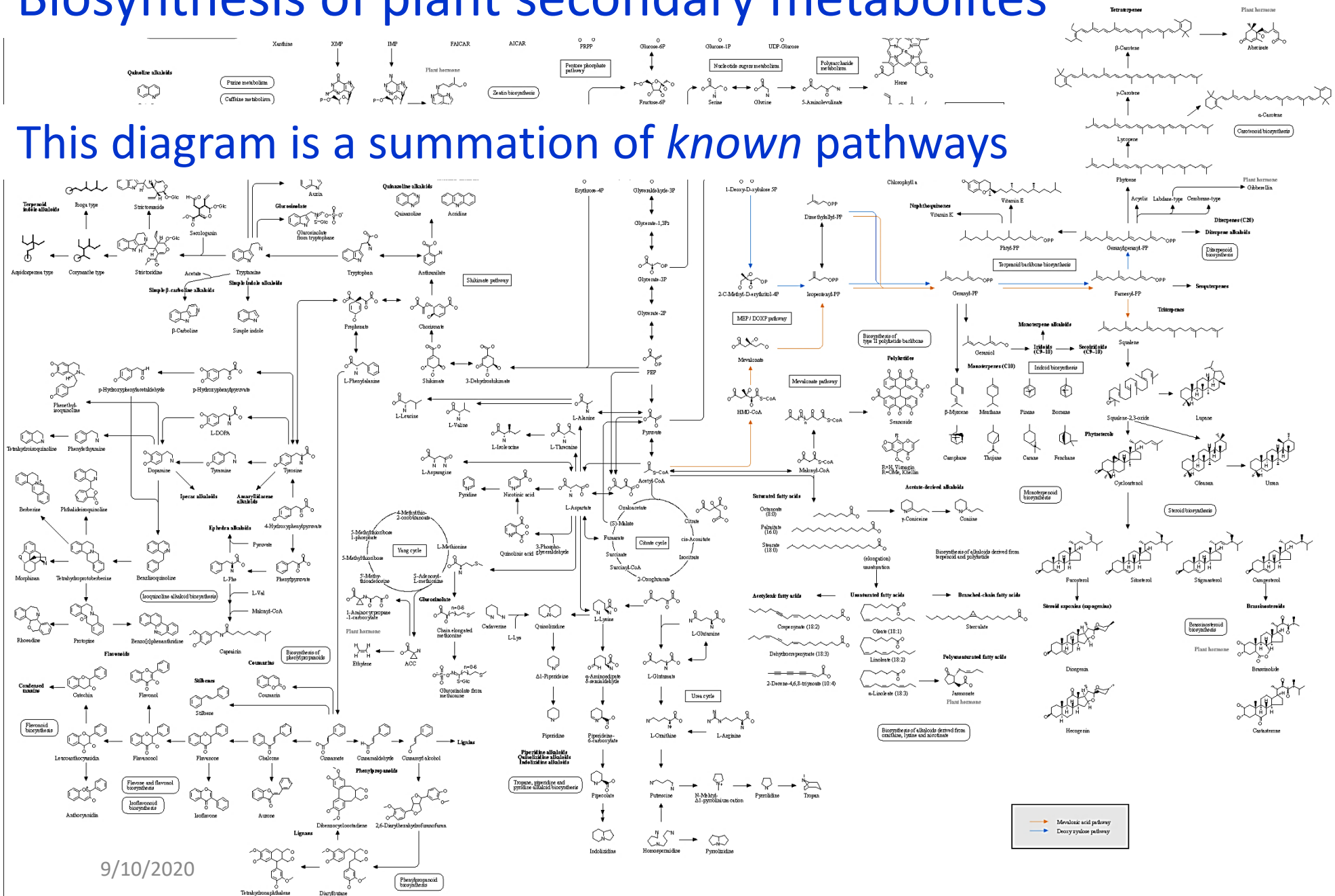
Camptothecin levels are highest in immature leaf tissue

A. Daniel Jones
Professor
Director, Mass Spec Facility

MICHIGAN STATE UNIVERSITY

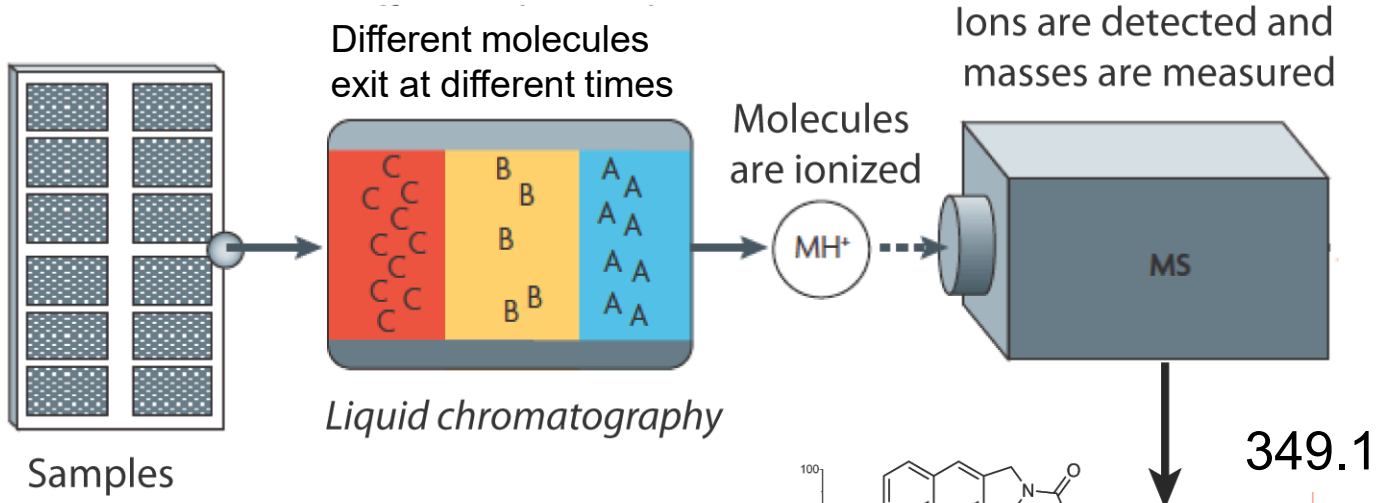
Biosynthesis of plant secondary metabolites

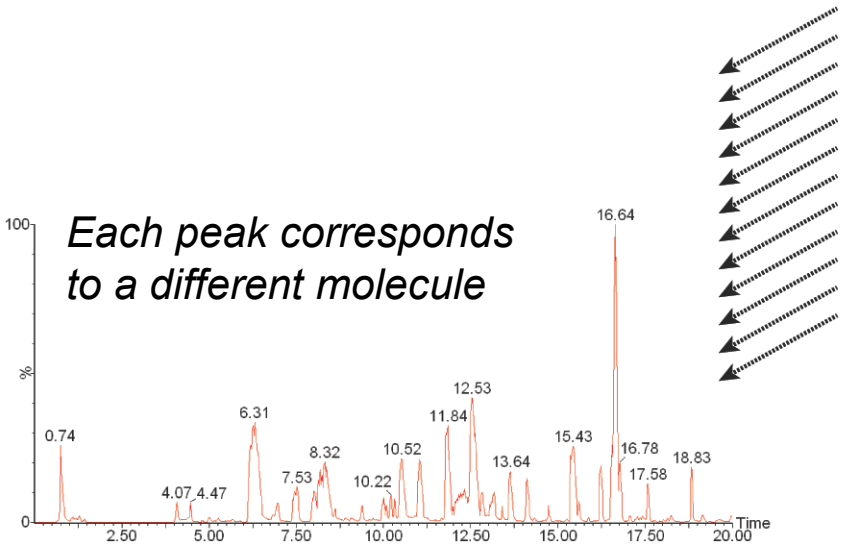
This diagram is a summation of *known* pathways



Metabolomics technology suggests that 80-90% of plant secondary metabolites are unknown compounds.

Separating the secondary metabolites in a plant tissue

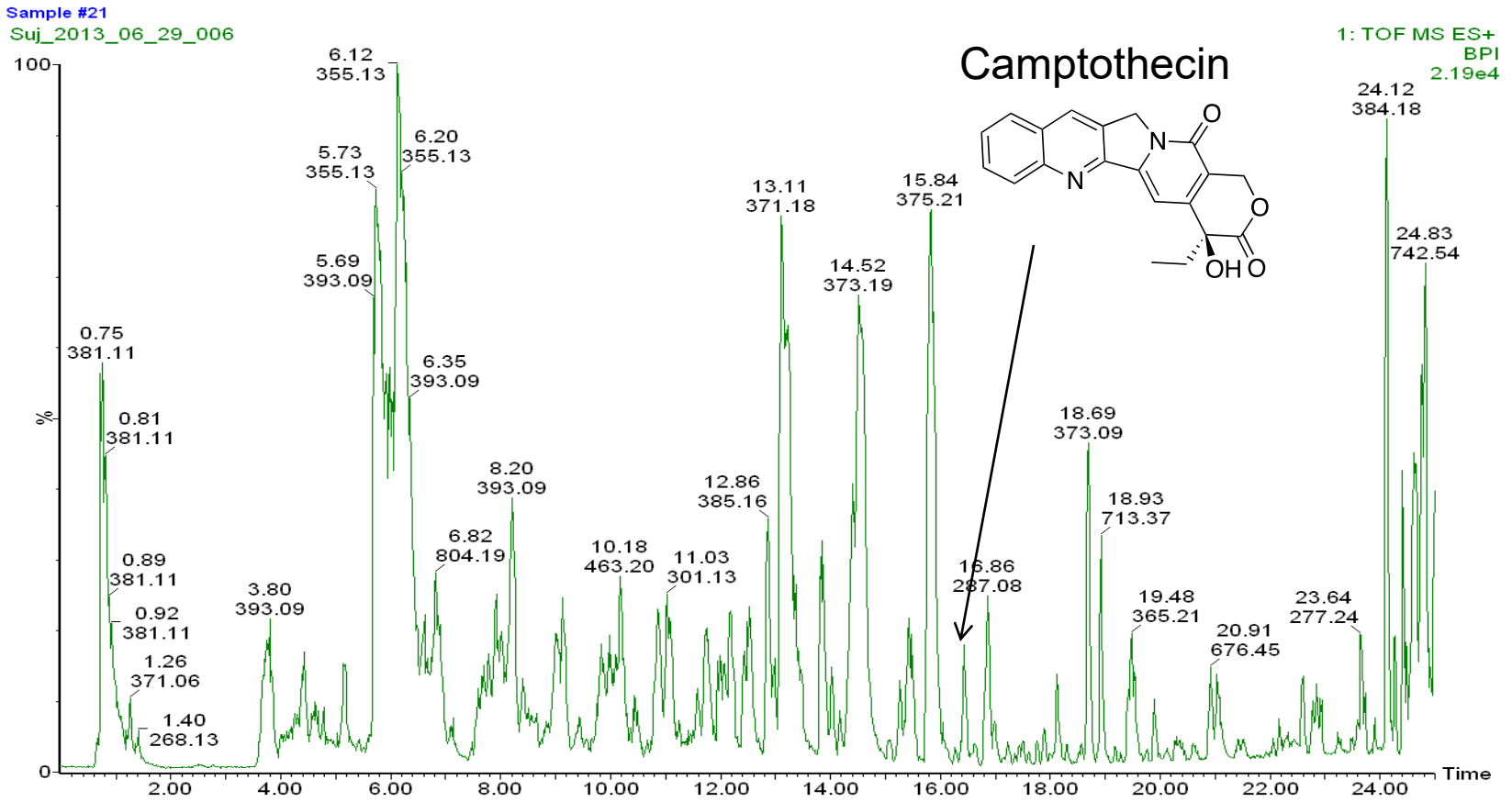




- Specialized equipment
  - Mostly automated once the tissue is extracted
- Specialized software
- Analytical chemists
- Statistician

Camptotheca acuminata

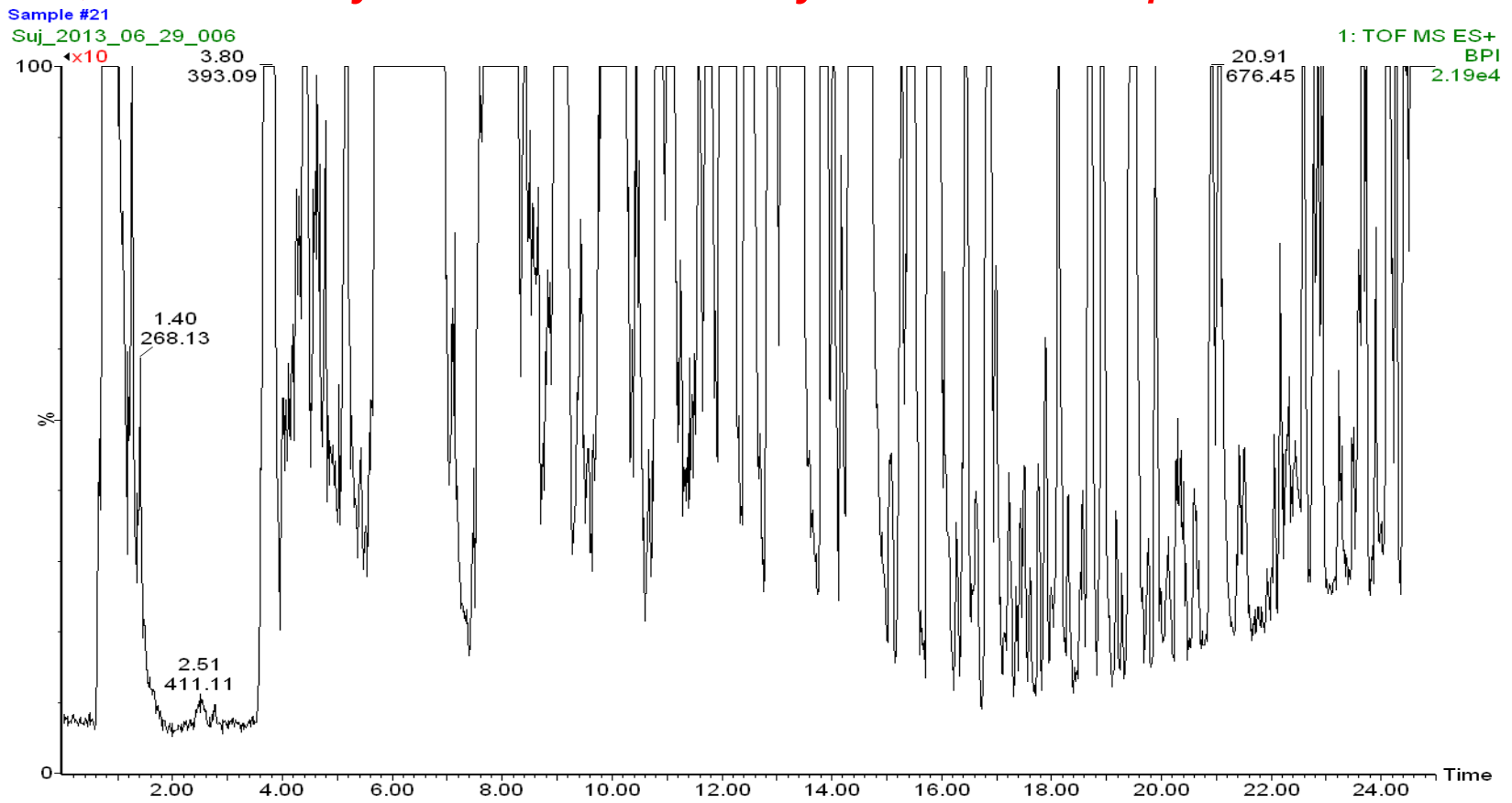
How many metabolite peaks do you see?



Sujana Pradhan
Maria Magallanes-Lundback

LC/MS profile magnified 10 X

Now, how many metabolites do you think are present?



Evidence for more than 1000 metabolites

Sujana Pradhan
Maria Magallanes-Lundback

Untargeted metabolomics workflows

- Have you defined the scientific question?
- Can you answer the scientific question with the host material available?
- Have you done preliminary studies?
- Do you have direct access to the biological samples at the right time?
- Do you know what the right time is?
- Have you decided or do you know about storage, extraction, and metabolite stability?
  - No single solvent will dissolve all metabolites
- Choose analytical method
- Choose analytical data collection method
- Understand the basics of the data processing and statistical analysis
  - Do you understand what you are seeing?
- Search Databases to see if your compounds match anything known
  - Match not required for usefulness
- Validation of results

→ Credible biological interpretation

How to make metabolomics work for you
Breeding for stress resistance and/or host-pathogen interaction

1) Understand the sources of phenotypic variation and design to detect or avoid sources of variation from the start.
2) Know what your goal is and stick to it
   A. **Host plant breeding program:** Development of American chestnut or its ecological equivalent with enough resistance to ink disease and chestnut blight and enough genetic diversity to maintain self-sustaining populations.
   B. **Genetic mechanisms:** Elucidation of the precise genetic mechanisms of host-pathogen interactions so that we can use transformation to insert a corrective gene or CRISPR technology to edit the genome.

A real example of the use of metabolomics with a type A goal

- Goal
  - **Primary:** Development of green ash with enough resistance to EAB and enough genetic diversity to maintain self-sustaining populations.
  - **Secondary:** Development of a field test kit that will reveal the presence of metabolites diagnostic for high larval kill

- Progress towards goal*
  - **Primary:** we have full sib progeny with larval kill as good as Manchurian ash (based on EAB egg bioassays)
  - **Secondary:** we have a set metabolites that correctly identify 70-80% of the trees with high larval kill, 70% of the low larval kill trees and incorrectly identify none. **$$$$$**

*** Requires confirmation in replicated studies across years, work is in progress**

A reality check

EAB resistance breeding in green ash: 18 years from detection in Detroit, 2002

- Year 5-present :identify lingering ash in monitored forest plots first monitoring plots established in 2004, first data collected 2005, first lingering ash propagated in 2008-9 – so if you want to use 2008 that would be year 6.
- Year 7-13 : develop and refine reproducible EAB infestation and stem dissection procedures
- Year 6 to present: produce grafted clonal replicates of lingering ash for replicated tests
- Year 8 to present: make crosses between the best lingering ash parents
- Year 14 to present: phenotype full sib families large enough for power of test and seek funding for Omics
- Years 14-18: Do the transcriptome and metabolome of full sib families and their parents, of the right tissue (inner bark, both cambiums, sapwood) taken at the right time (8 weeks after infestation) at the right age**.....work in process**
- Years 18 and on: test the predictive values of the group of metabolites identified and if confirmed, develop a diagnostic test for high larval kill in trees artificially infested and those under attack in naturally regenerated stands**.....work in process**

EAB resistance breeding team

Multiple disciplines, multiple institutions
Long term commitment

University of Notre Dame

Jeanne Romero-Severson, PhD.
Quantitative genetics and genomics

Robert K. Stanley,
PhD candidate
Analytical chemistry, Metabolomics

Michigan State

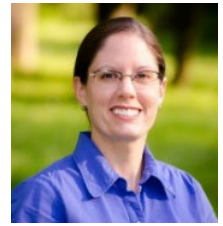
A. Daniel Jones, PhD.
Biochemistry, analytical chemistry, metabolomics

Lead Institution

US Forest Service
Northern Research Station

At Delaware, OH

Jennifer Koch, PhD.
Resistance breeding, Species restoration



Kathleen Knight, PhD.
Restoration ecology, invasive pests and diseases

At East Lansing, MI

Therese Poland, PhD.
Forest entomology
East Lansing, MI

Funding agencies

United States Department of Agriculture
Forest Service

United States Department of Agriculture
Animal and Plant Health Inspection Service

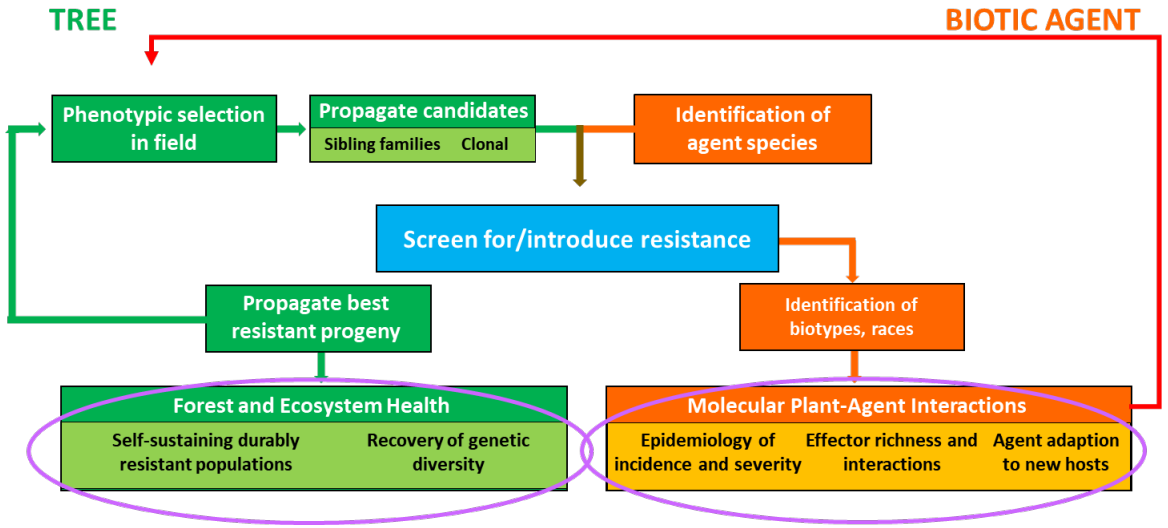
The Pennsylvania Department of Conservation and Natural Resources

The Chemistry-Biochemistry-Biology Interface (CBBI) Program at Notre Dame NIH training grant

TACF use of omics
Both Type A and Type B goals

A. Development of American chestnut or its ecological equivalent with enough resistance to ink disease and chestnut blight *and* enough genetic diversity to maintain self-sustaining populations.

B. Elucidation of the precise genetic mechanisms of host-pathogen interactions so that we can use transformation to insert a corrective gene or CRISPR technology to edit the genome.



Best practices for the development and deployment of improved pest and pathogen defenses in forest trees

- Professional guidance
  - Plant breeding, Quantitative genetics, Statistics and experimental design, Silviculture, Quality control, Analytical chemistry, Bioinformatics, Other Omics expertize, Project management
- Clear goals, regularly reviewed
- Long term commitment

Useful resources

Metabolomics Association of North America

https://metabolomicsna.org

Metabolomics Society

http://metabolomicssociety.org/