Metabolomics approaches for tracking biotic and abiotic stress performance in tree improvement programs

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Outline

- An introduction to metabolite-based screening
- Evaluating chemical fingerprinting as a tool to screen chestnut for disease resistance
- Targeted metabolomics to track developmental progression in peach and apricot
- Conclusions

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Plant secondary metabolites (PSMs)

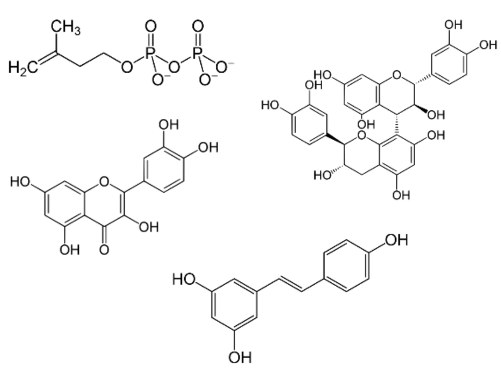
- PSMs in particular are known for their association with tree stress response
- Types and amounts are tree-species dependent
- Temporally and spatially variable

Chemical biomarkers

- Certain chemicals may be associated with a trait of interest
  - e.g. disease resistance/susceptibility

- Goal is to identify chemicals that may serve as biomarkers

- In some cases, statistical models can be developed to predict the trait based solely on a tree’s chemical composition



Images from: http://www.wikipedia.org

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https://www.srs.fs.usda.gov/uplandhardwood/research-topics/duplicates/american-chestnut.html

Metabolite-based screening approaches

- Identify and quantify specific metabolites or measure general profiles
- Different tools for different objectives/questions
- Examples of different analytical approaches include:
  - FT-IR spectroscopy
  - HPLC-MS

http://iss-store.co.uk/catalog/chromatography-systems/waters-chromatography-systems/waters-lc-ms-systems

Overall objectives

- Evaluate general metabolomic profiles for the identification of chemical fingerprints linked to pathogen resistance in American x Chinese chestnut hybrids

- Evaluate specific intermediates within the phenylpropanoid pathway as biomarkers for developmental progression linked to annual climatic cycling in fruit trees

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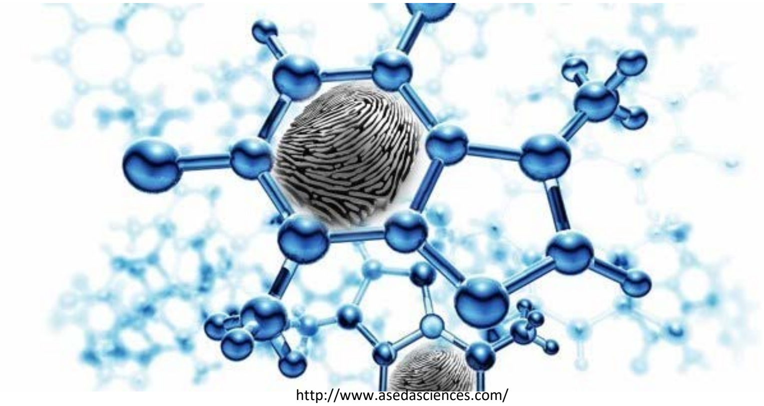
What is chemical fingerprinting?

- **Chemical fingerprinting** is a comprehensive analysis of all the chemicals present within a given sample

- Individual chemicals are not separated, identified, or measured

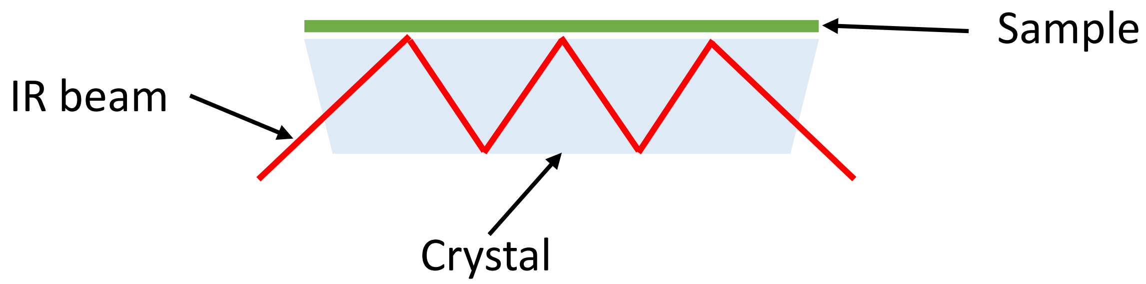
- Provides a “snap-shot” of the chemical composition of a given tissue at a given time



http://www.asedasciences.com/

Fourier transform infrared spectroscopy

- Fourier transform infrared (FT-IR) spectroscopy is one method of chemical fingerprinting
- FT-IR spectroscopy measures how a sample absorbs light over a wide spectral window
- Differences in chemical composition/concentration will affect the FT-IR spectrum



FT-IR spectrometers

Benchtop spectrometer

“Portable” spectrometer

Handheld spectrometer

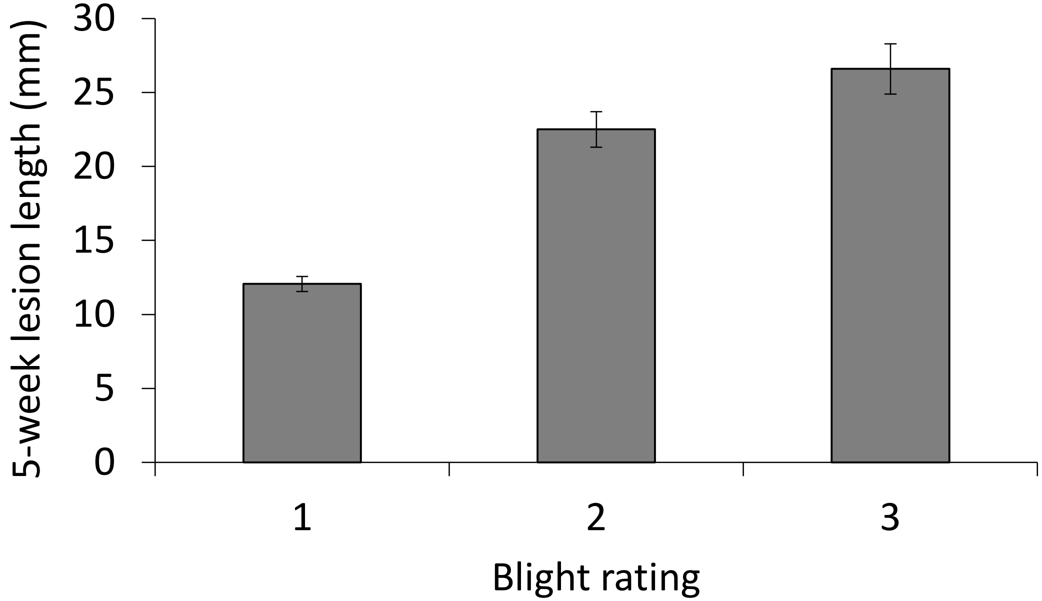
Photo credits: (left) http://www.agilent.com/en-us/products/ftir/ftir-benchtop-systems/cary-660-ftir-spectrometer; (right) https://www.agilent.com/en-us/products/ftir/ftir-compact-portable-systems/4100-exoscan-series-ftir-(handheld)

Chestnut chemical fingerprinting

Evaluate if chemical fingerprinting can be used to screen hybrid chestnut for disease resistance prior to infection with chestnut blight or Phytophthora root rot

Photo credit: Linda Haugen, USDA Forest Service, Bugwood.org

Phenotypic variation is necessary to build predictive models for disease resistance

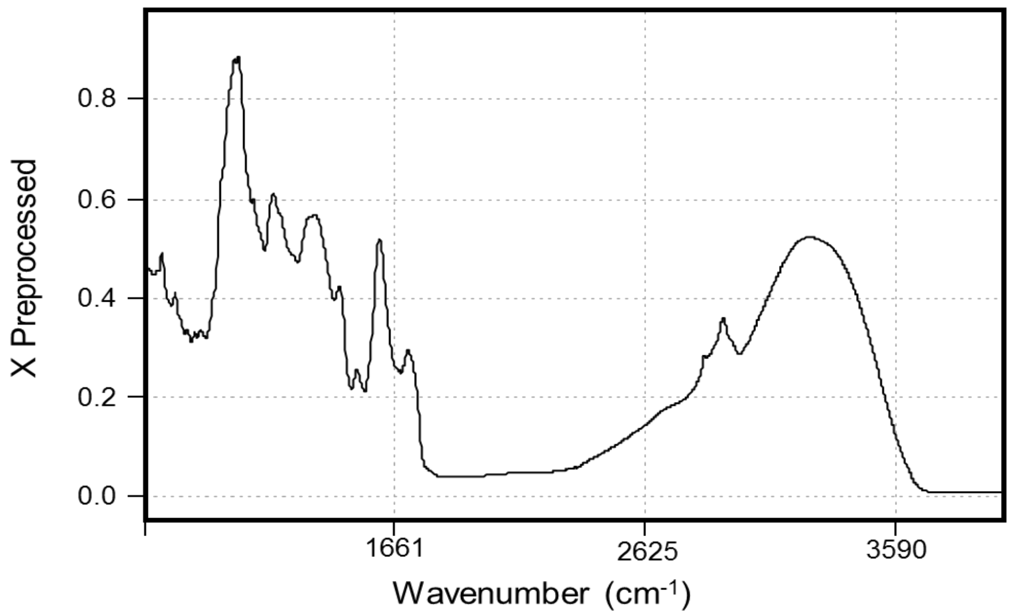


Average (± standard error) lesion length of BC_3F_3 hybrids derived from Clapper for each blight rating group (N = 41). Phenotypic data and tissue samples provided by J. Westbrook (TACF).

Chestnut chemical fingerprints

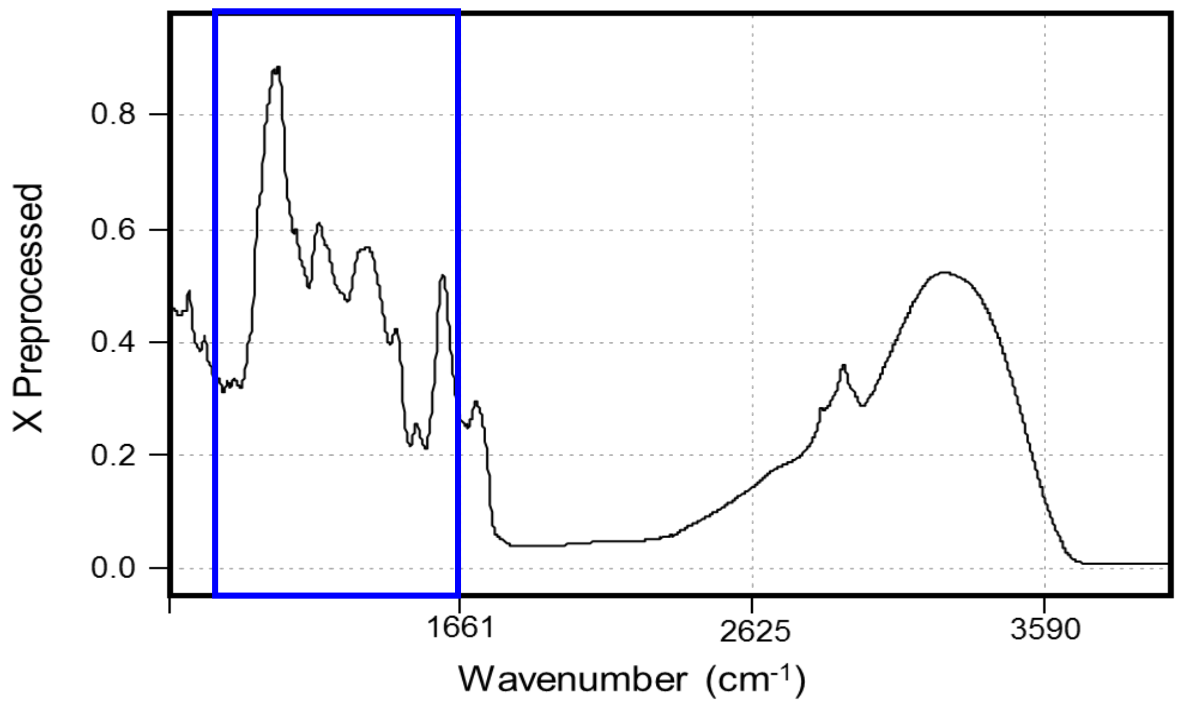
Benchtop
FT-IR spectrometer

http://www.agilent.com/en-us/products/ftir/ftir-benchtop-systems/cary-660-ftir-spectrometer



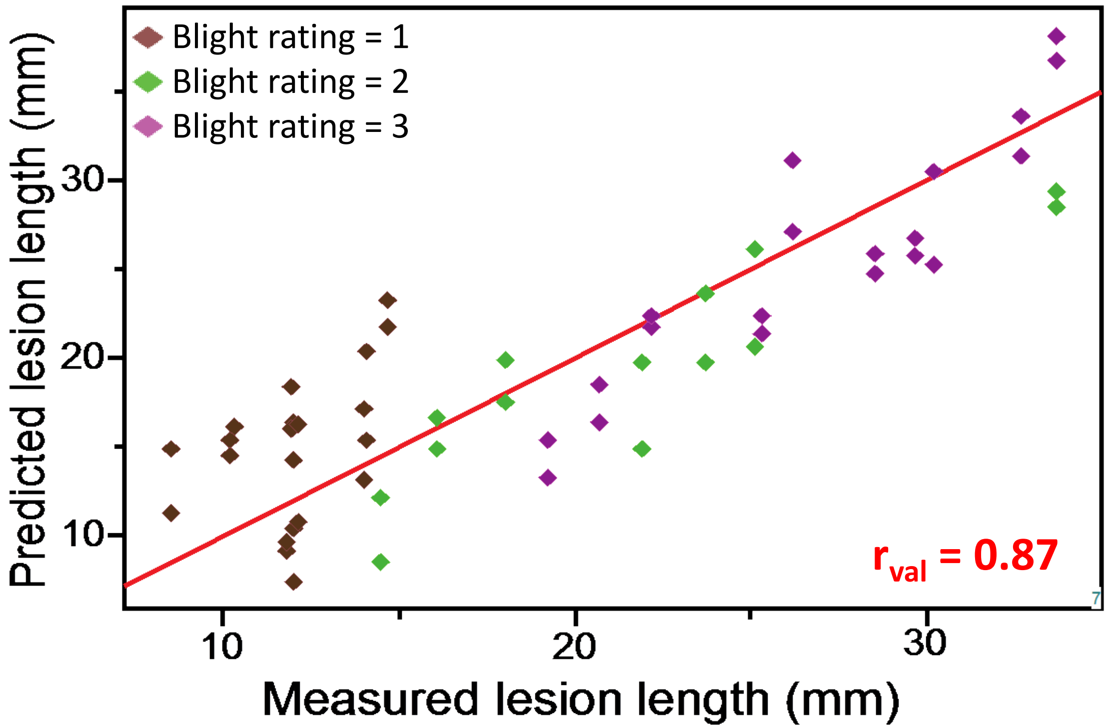
A representative hybrid chestnut spectrum collected from the mid-infrared region.

Focus on a specific spectral region



For blight analysis, focused on spectral range: 901 – 1622 cm^{-1}.

Susceptibility of ‘Clapper’ hybrids to blight can be estimated using spectral data



Correlation plot from 7-factor partial least squares regression analysis of Clapper data set showing the relationship between measured and predicted lesion lengths, based on transformed spectral data. N = 55, with 2 technical replicates per biological replicate and outliers trimmed based on preliminary analysis.

Application of chemical fingerprinting

- Chemical fingerprinting has the potential to be a useful tool for screening hybrid chestnut for disease resistance
- Additional evaluations are planned for summer 2017
- Potential for more high-throughput analysis in the field/forest

Handheld Raman Spectrometer

http://www.rigakuanalytical.com/

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Developmental progression and adaptive traits

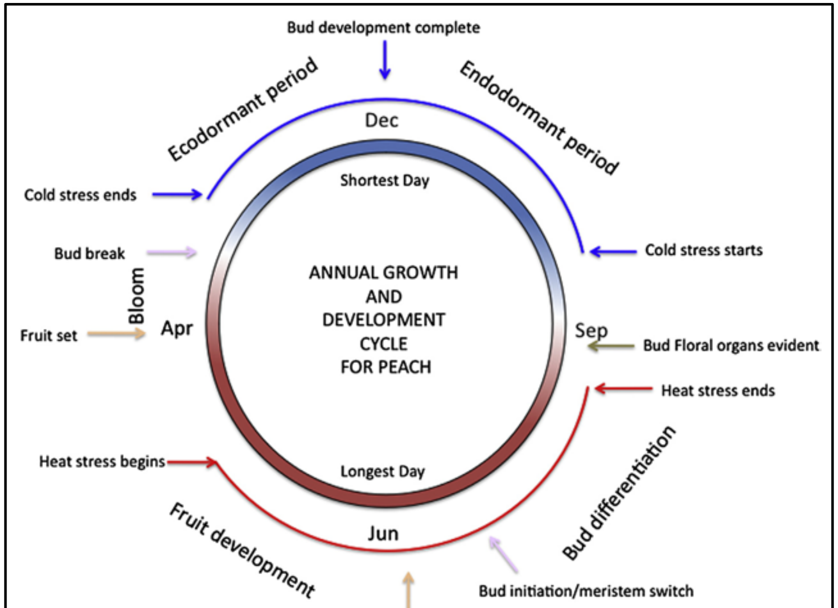
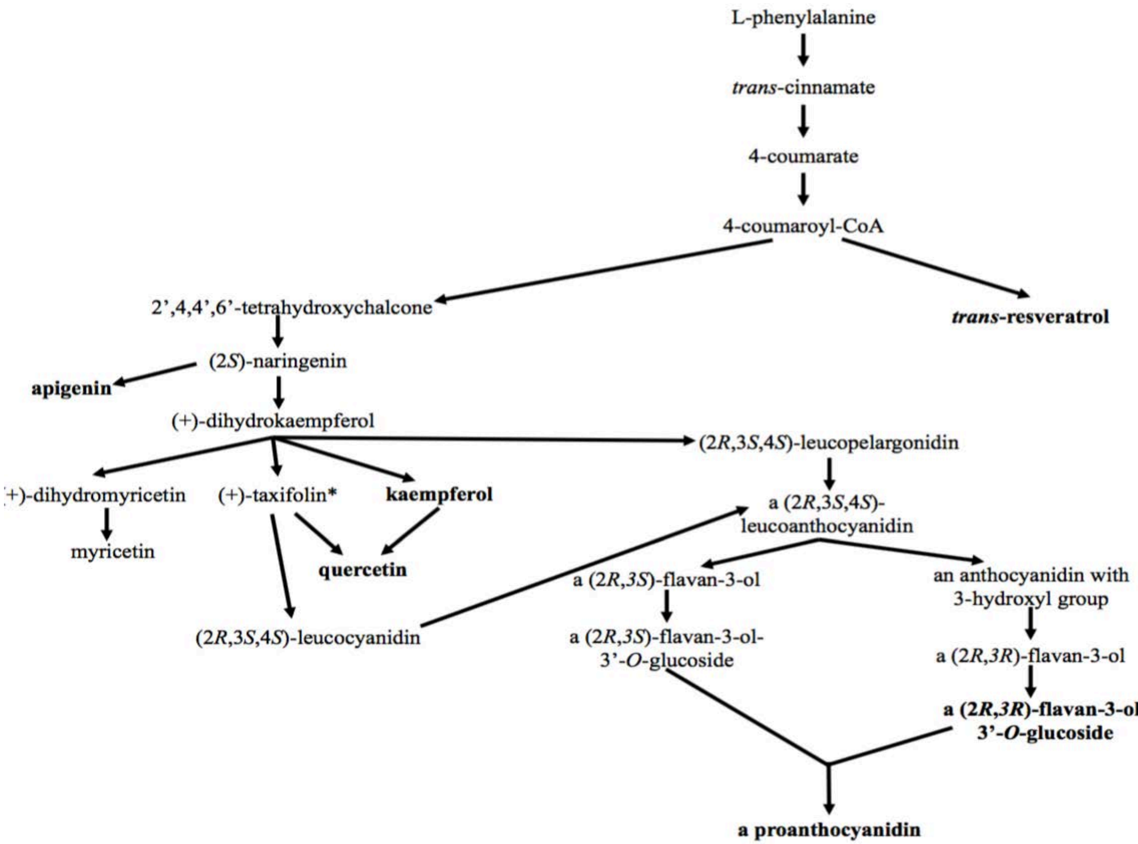


Figure from Abbott et al. 2015

- Phenology is the study of cyclic events and the influence of climatic and seasonal variability on these events
- Dormancy, bloom date, and chilling requirement are examples of adaptive phenological traits
- Trait variability may be of interest to breeding programs

Identifying chemical markers for developmental progression

Examine the association between adaptive trait variation and phenylpropanoid intermediates in dormant floral buds



Simplified phenylpropanoid biosynthetic pathway in peach.

Adaptive traits

Species	Phenotype	Variety/Individual
Peach	Low-chill	A209
	Low-chill	A340
	High-chill	A318
	High-chill	A323
Apricot	Early flowering	A2312
	Early flowering	A2137
	Early flowering	A1956
	Late flowering	A660
	Late flowering	A1267

Photo credit: (TOP) Carroll E. Younce, USDA Agricultural Research Service, Bugwood.org; (BOTTOM) Howard F. Schwartz, Colorado State University, Bugwood.org

Targeted metabolomics analysis

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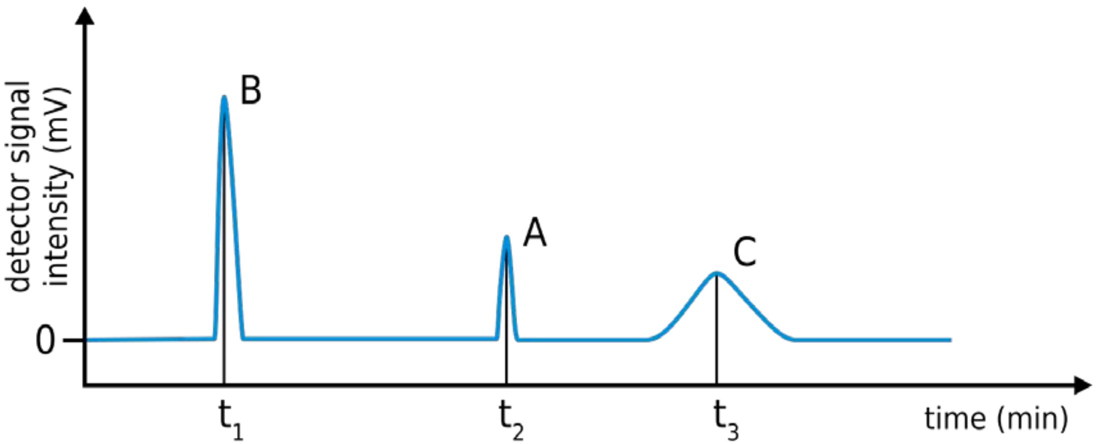
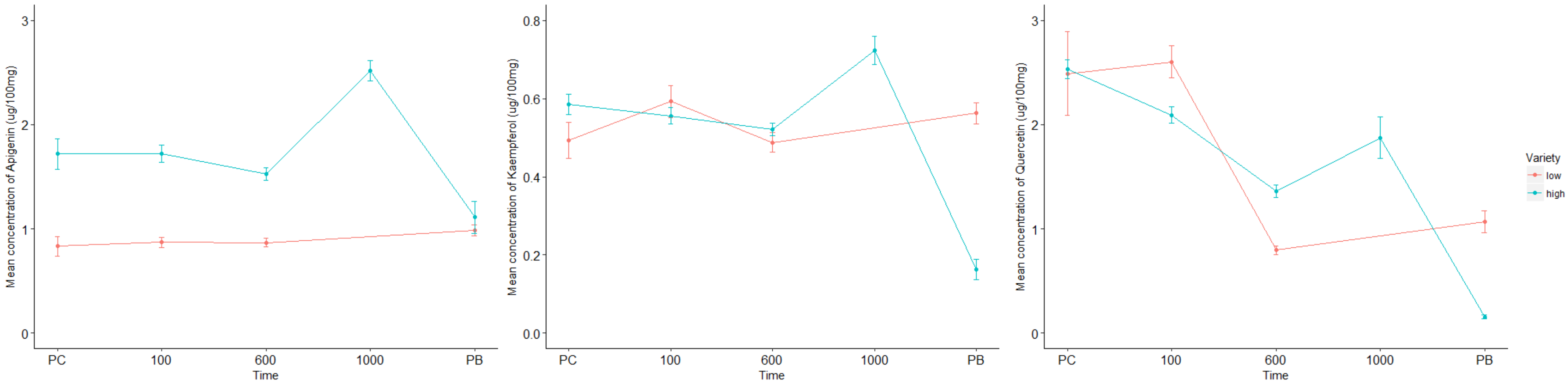


https://commons.wikimedia.org/wiki/File:Chromatogram_in_English.svg

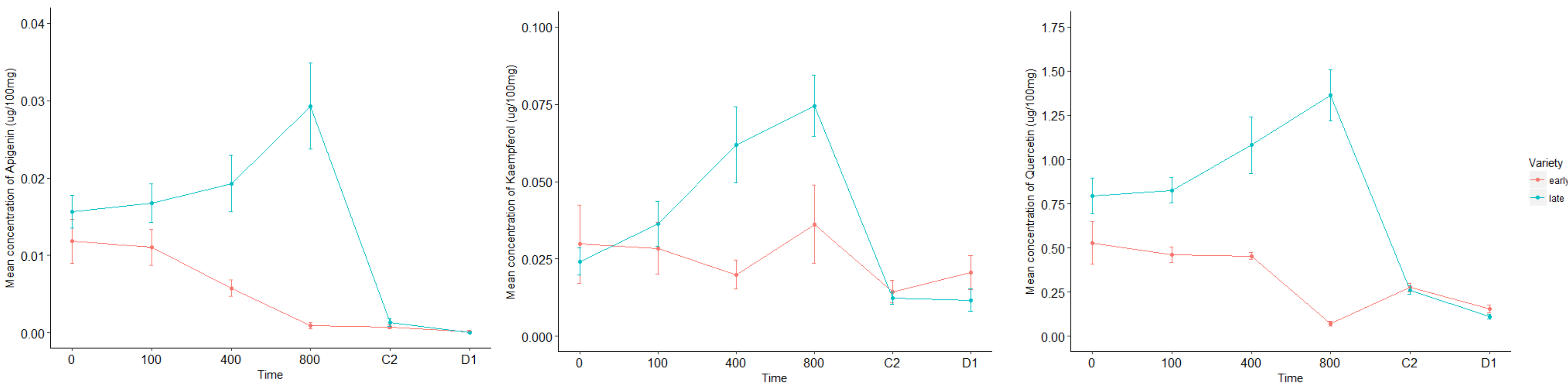
Flavonoids: apigenin, kaempferol, quercetin
Flavan-3-ols: epicatechin-3'-*O*-glucoside; Procyanidin B1, B2, B3
Stilbene: resveratrol

Flavonoid concentrations change during dormancy and are impacted by tree genotype in peach



Concentrations of flavonoid aglycones, apigenin, kaempferol, and quercetin, were significantly affected by the interaction of time (at 100, 600, and PB) and chilling requirement (i.e. genotype). Repeated measures ANOVA ($P < 0.05$).

A similar pattern is observed in apricot



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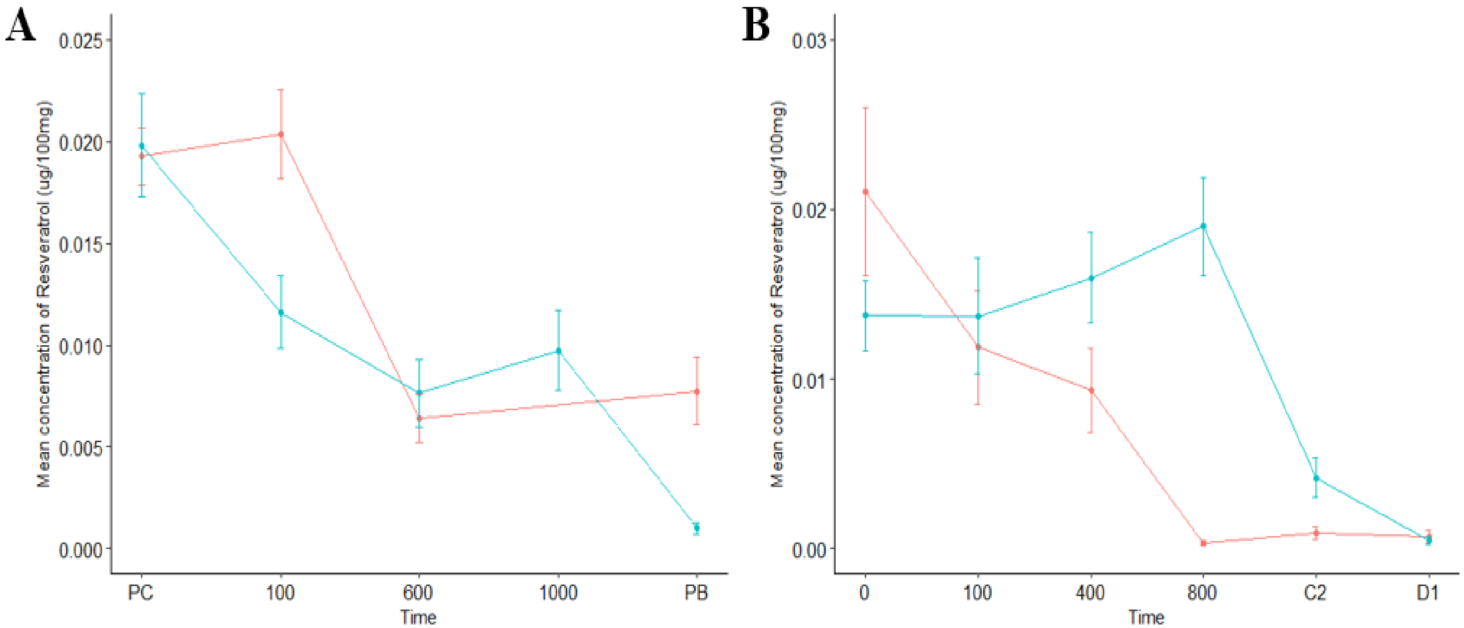
Phenylpropanoids as biomarkers for developmental progression

- Concentrations of phenylpropanoid intermediates change as dormancy progresses

- Pronounced differences in concentration between endo-dormant and post-dormant buds

- Timing of changes is impacted by phenotype/genotype

Changing in response to environmental cues?



Resveratrol in peach (**A**) and apricot (**B**) buds during dormancy in trees that varied in chill requirement (CR) and bloom date (BD). **Red**: low. **Blue**: high.

Concentrations of resveratrol are known to be impacted by changes in water availability

Application of chemical biomarkers

- Alternative method for determining developmental stage
- Predict low versus high chill genotypes/phenotypes

Photo credit: University of Georgia Plant Pathology , University of Georgia, Bugwood.org

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Conclusions

- Different metabolomics approaches can be used to generate chemical phenotypes (chemotypes) of trees
- Chemotypes may be associated with trait of interest
  - Disease resistance/susceptibility (e.g. chestnut)
  - Developmental progression (e.g. peach and apricot)
- Application for tracking biotic and abiotic stress performance in tree improvement programs

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