Below Ground Regulation of Stress Response Genes in Hybrid Poplar

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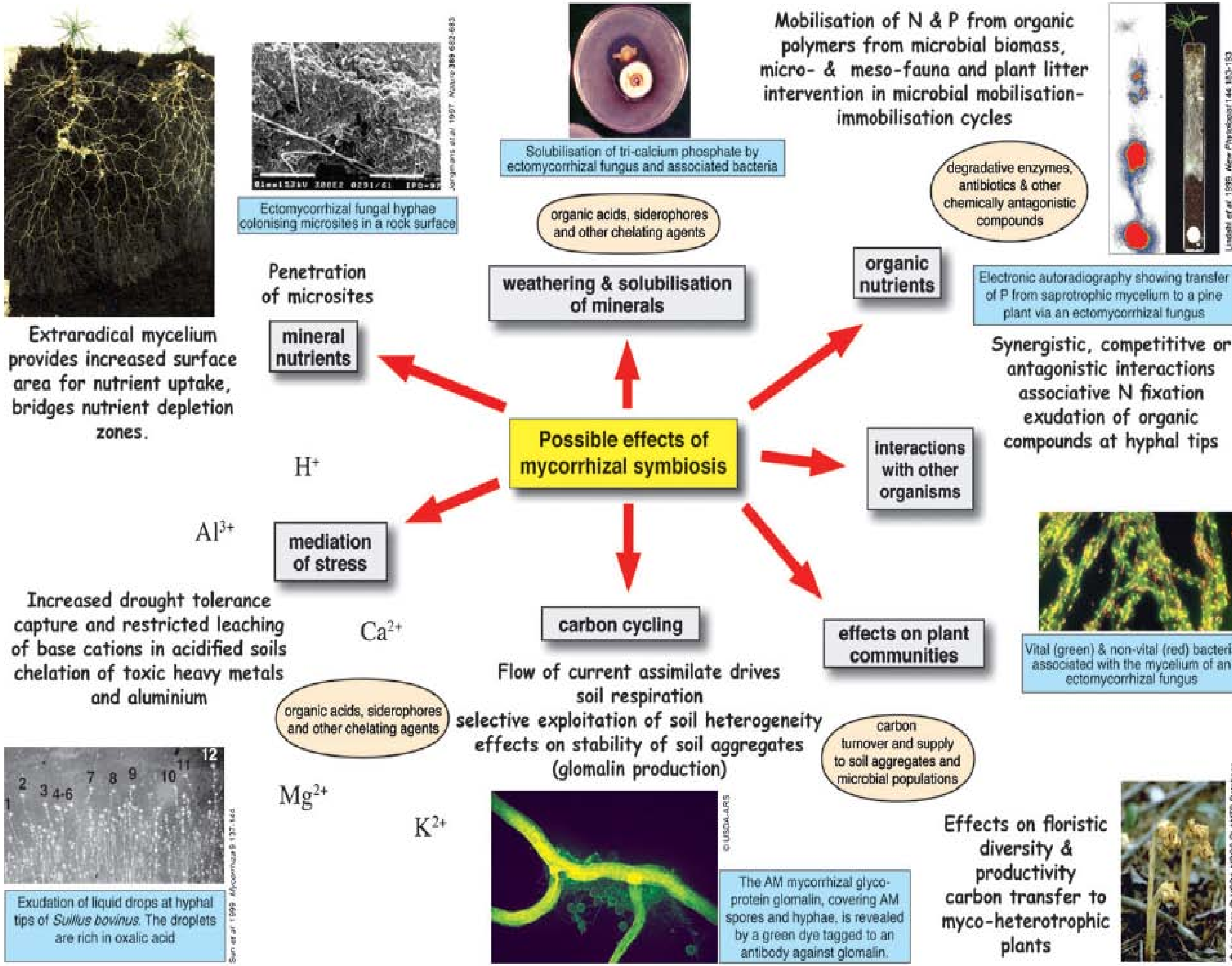
Presentation Outline

- Introduction to Below-Ground Fungal Associations of Trees
- Navigating Multiple Stress Responses in Hybrid Poplar
    - Abiotic (Ozone) and Biotic (Insect Herbivory)
    - Microarray and RT-PCR Gene Expression Changes
    - Understanding the Role of Jasmonic Acid in Stress Response
- Understanding Stress Responses in Hybrid Poplar Biomass Plots
    - Tree Spacing, Growth, and the Use of 1-MCP (ethylene blocking agent) in Stress Response
    - Soil Metagenomics of Poplar Biomass Plantations
- Final Conclusions and Acknowledgements

Truncocolumella on *Pseudotsuga* roots (Oregon).


Photo: David Read



Possible effects of mycorrhizal symbiosis
mineral nutrients
Penetration of microsites
Extraradical mycelium provides increased surface area for nutrient uptake, bridges nutrient depletion zones.
Ectomycorrhizal fungal hyphae colonising microsites in a rock surface
weathering & solubilisation of minerals
organic acids, siderophores and other chelating agents
Solubilisation of tri-calcium phosphate by ectomycorrhizal fungus and associated bacteria
organic nutrients
Mobilisation of N & P from organic polymers from microbial biomass, micro- & meso-fauna and plant litter intervention in microbial mobilisation-immobilisation cycles
degradative enzymes, antibiotics & other chemically antagonistic compounds
Electronic autoradiography showing transfer of P from saprotrophic mycelium to a pine plant via an ectomycorrhizal fungus
interactions with other organisms
Synergistic, competititve or antagonistic interactions associative N fixation exudation of organic compounds at hyphal tips
Vital (green) & non-vital (red) bacteria associated with the mycelium of an ectomycorrhizal fungus
effects on plant communities
Effects on floristic diversity & productivity carbon transfer to myco-heterotrophic plants
carbon cycling
Flow of current assimilate drives soil respiration selective exploitation of soil heterogeneity effects on stability of soil aggregates (glomalin production)
carbon turnover and supply to soil aggregates and microbial populations
The AM mycorrhizal glycoprotein glomalin, covering AM spores and hyphae, is revealed by a green dye tagged to an antibody against glomalin.
mediation of stress
H+
Al3+
Ca2+
Mg2+
K2+
Increased drought tolerance capture and restricted leaching of base cations in acidified soils chelation of toxic heavy metals and aluminium
organic acids, siderophores and other chelating agents
Exudation of liquid drops at hyphal tips of Suillus bovinus. The droplets are rich in oxalic acid
1 2 3 4-6 7 8 9 10 11 12

Finlay 2008

Fig.1 – Mechanism for possible role in defense priming from ectomycorrhizal fungal infection.

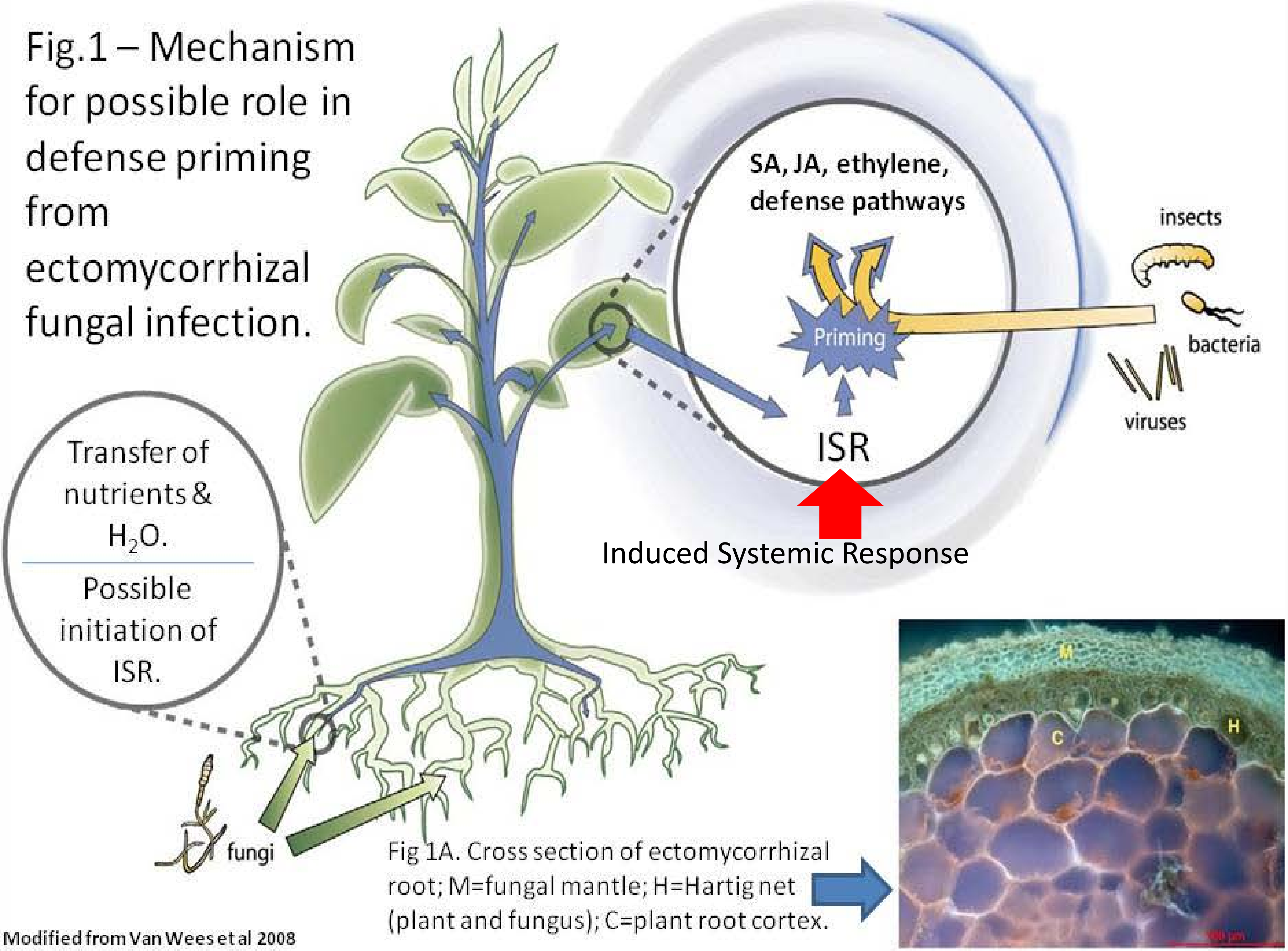


Fig 1A. Cross section of ectomycorrhizal root; M=fungal mantle; H=Hartig net (plant and fungus); C=plant root cortex.

Modified from Van Wees et al 2008

Presentation Outline

- **Introduction to Below-Ground Fungal Associations of Trees**

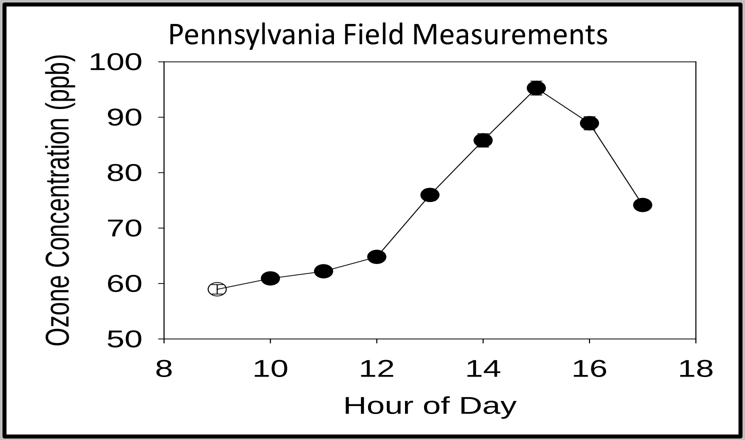
Original Goal of the Study was to address the role of Ectomycorrhizal Fungi in mediating responses to both Ozone (Teo Orendovici-Best) & Insect Herbivory (Christopher Frost). I found that mycorrhizal root quantification and fungal volatiles may have been problematic.

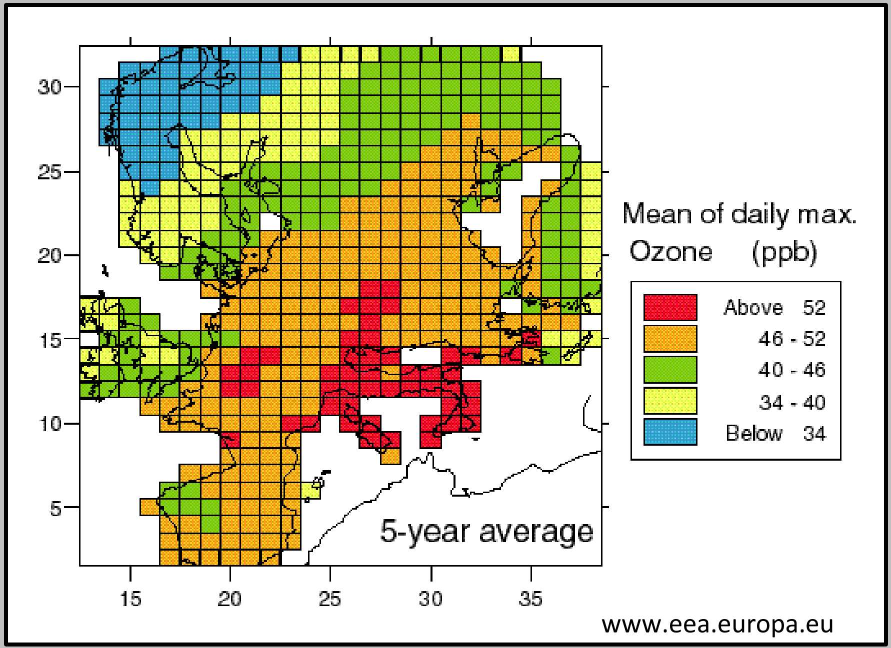
  - **Tree Spacing, growth, and the Use of 1-MCP (ethylene blocking agent) in stress response**
  - **Soil Metagenomics of Poplar Biomass Plantations**
- **Final Conclusions and Aknowledgements**

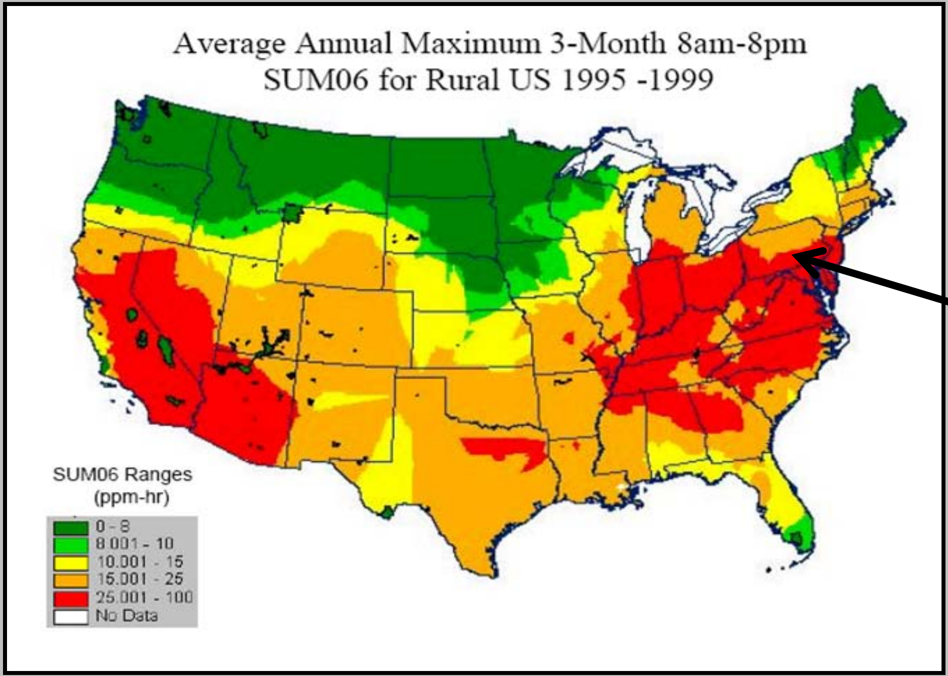
Ozone Study Outline

- **Introduction**
  - **Brief Introduction to Ozone Pollution**
  - **Our Observations and Preliminary Data Regarding Ozone Toxicity**
- **Methods**
  - **Interactions of Ozone Toxicity and Herbivory**
  - **Experimental Design**
- **Results**
  - **Herbivore-Ozone Transcriptome Changes in *Populus***
  - **Microarray and RT-PCR Gene Expression Changes Associated with the Jasmonic Acid (JA) Pathway**
  - **Functional Studies and Phylogenetics of JA Pathway Genes, a Unique Biochemical Bottleneck, in *Populus***

Ozone stress







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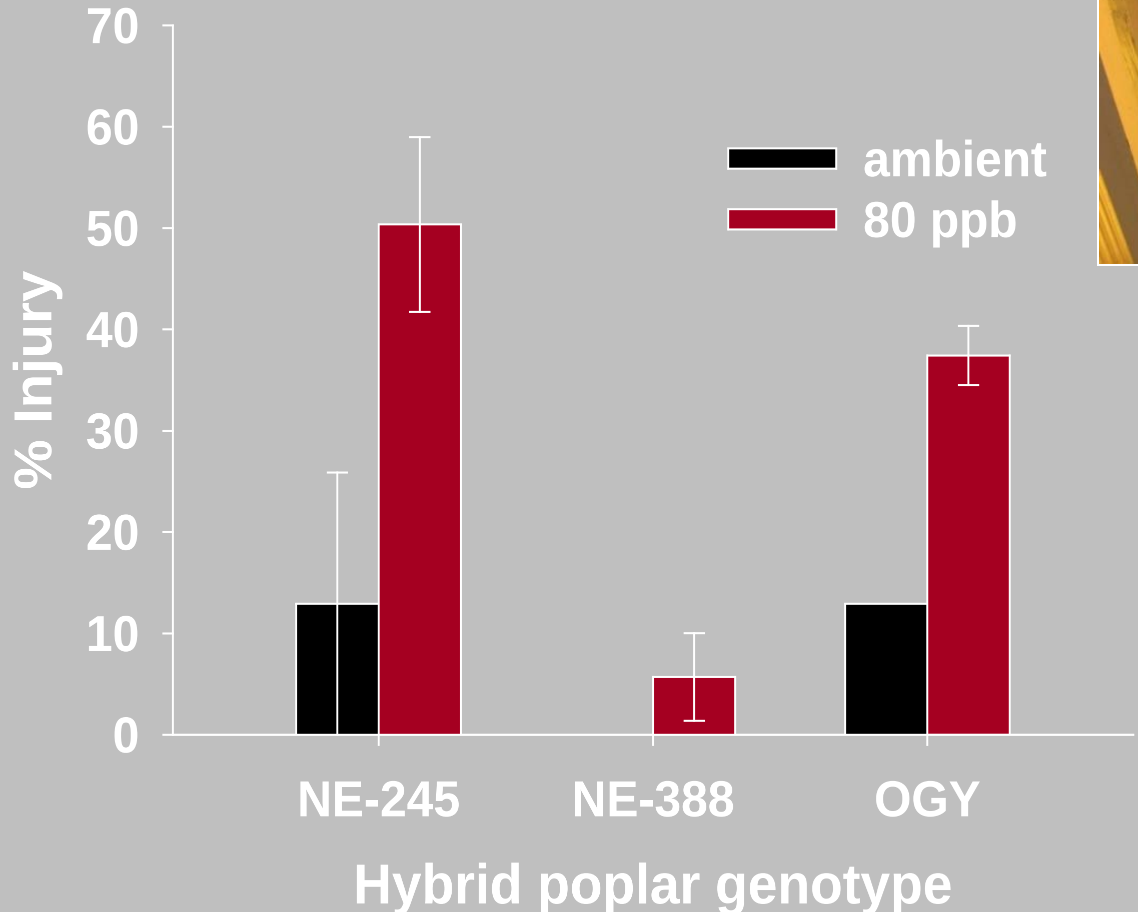
From: Nicholas, N.S., P.F. Brewer and D.A. Weinstein. 2000. *An Assessment of Ozone Effects in the Southern Appalachians Using a Multi-Stakeholder Process*. Paper 463. Presented at the Air and Waste Management Association Annual Meeting, June 18-22, Salt Lake City, Utah.

Populus genotypes can be sensitive to Ozone in the field

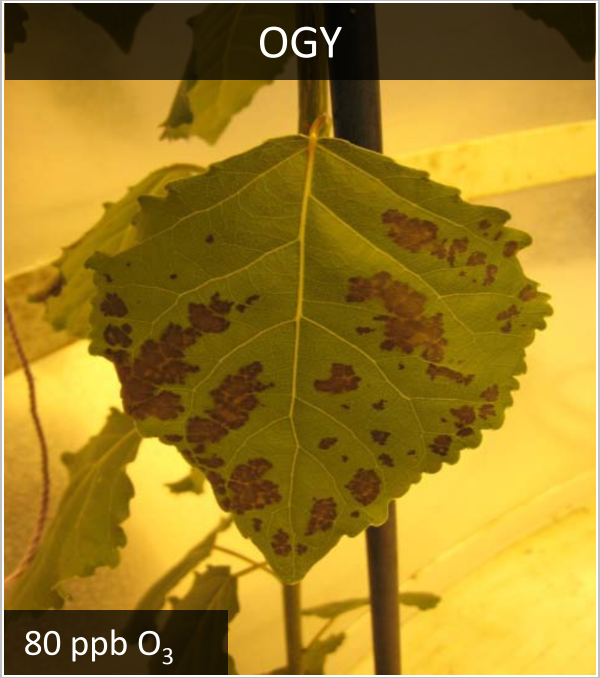
Populus Hybrid NE-245

Increasing Ozone concentrations

Genotypic variation in susceptibility to ozone



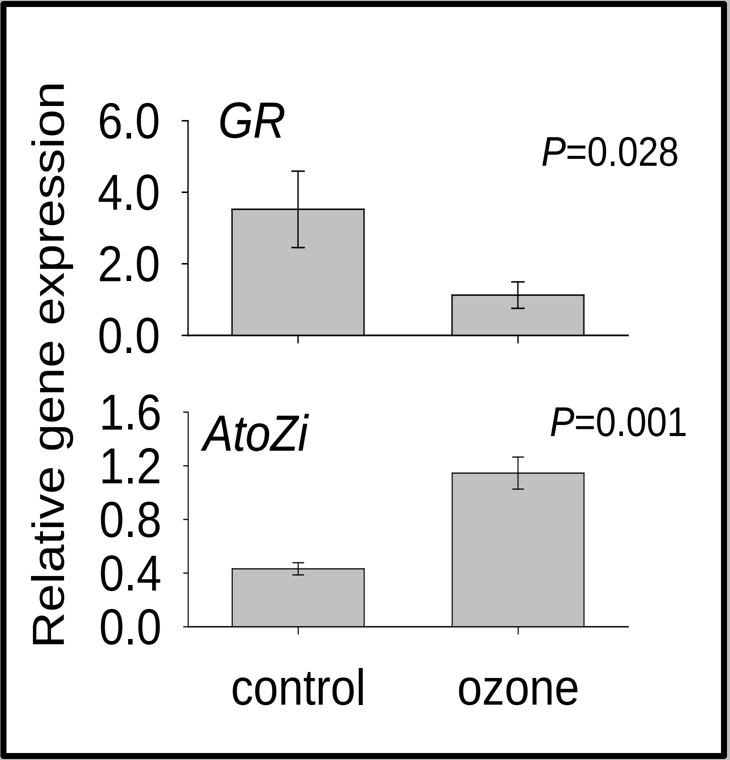
Orendovici-Best *et al.* (submitted)

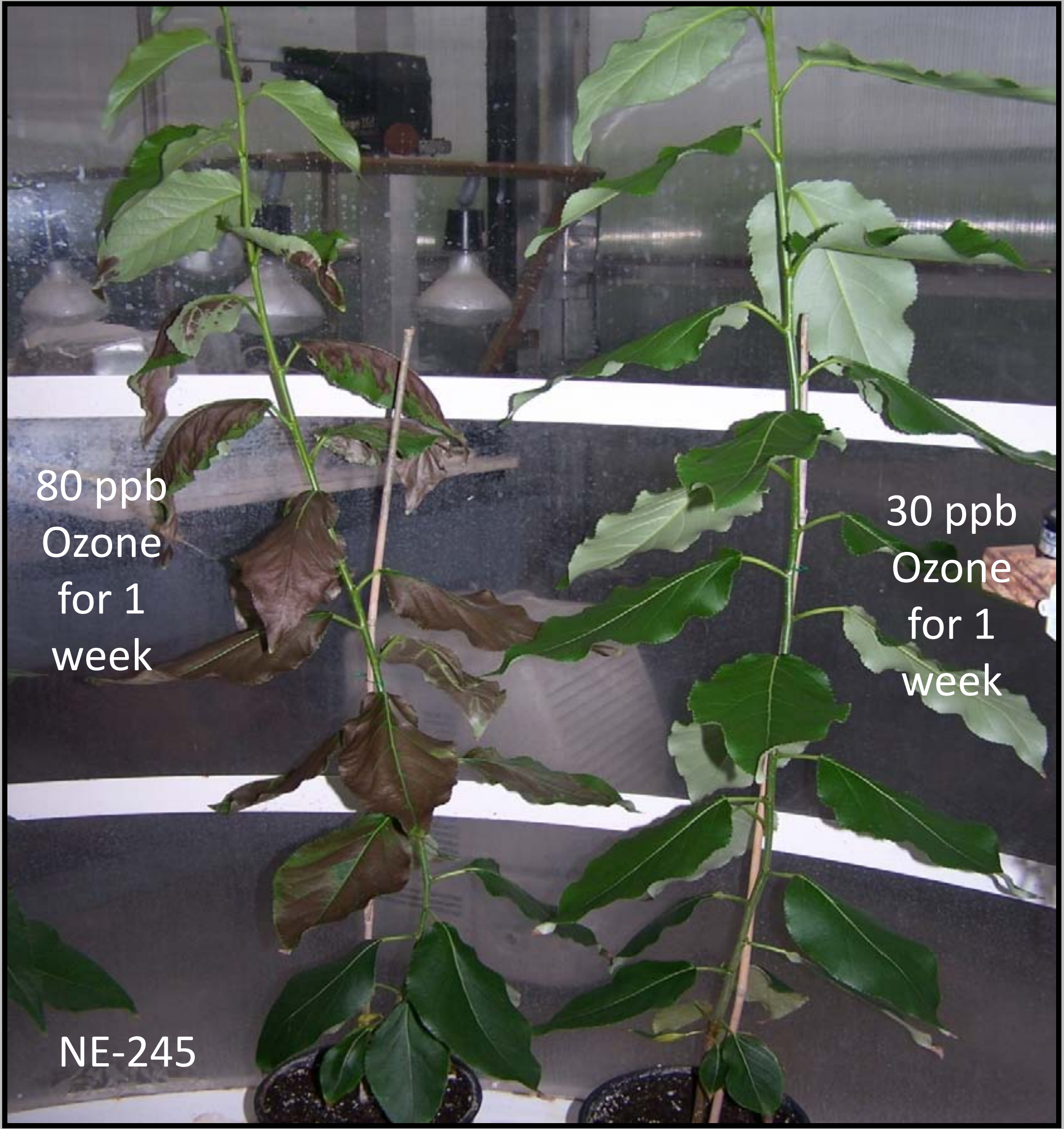


Ozone Induced Gene Expression Changes – Initial Observations

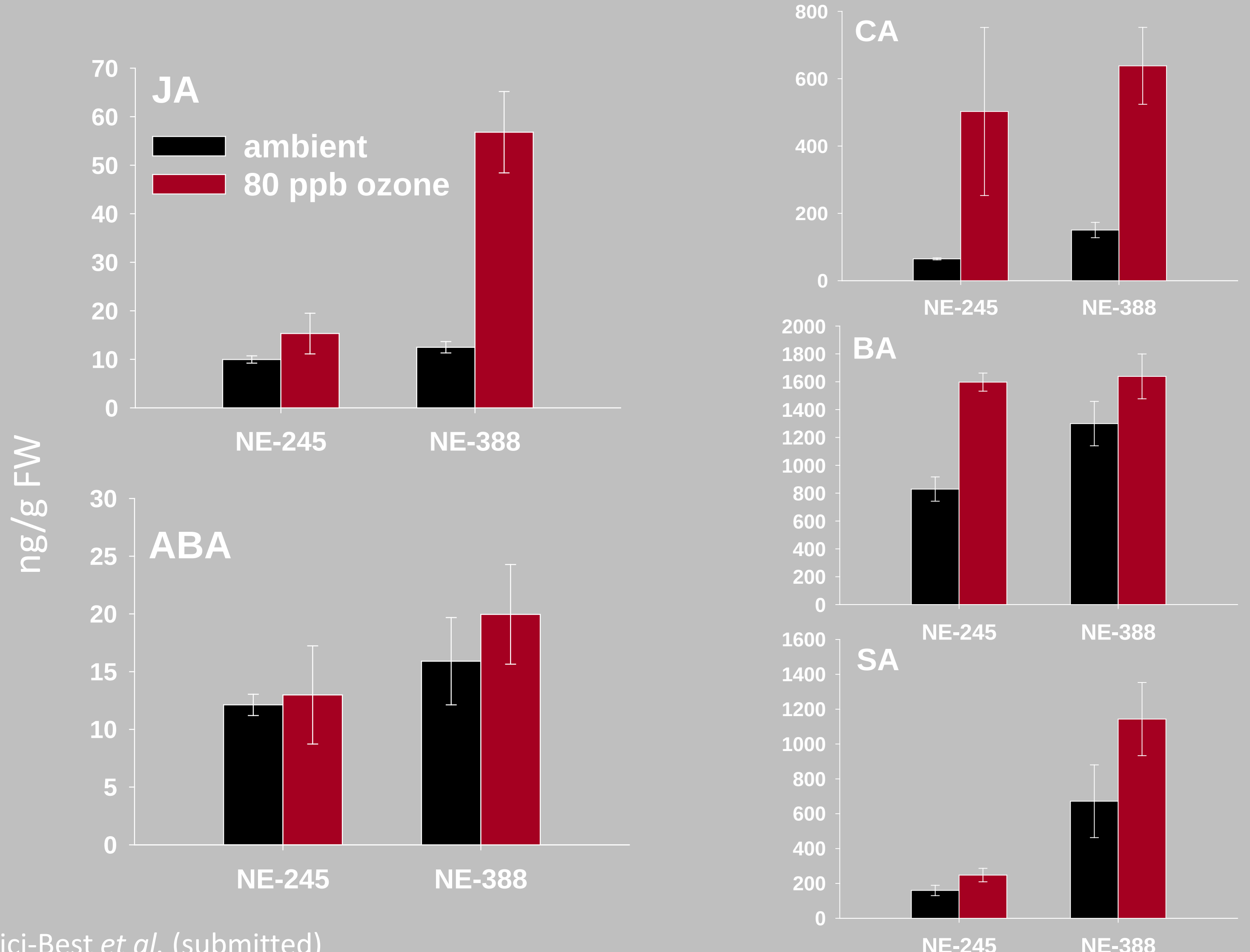
- **Populus hybrid NE-245 highly suceptible to ozone fumigation**

- **Decrease in *GR* (Glutathione Reductase) gene expression, Increase in *AtoZi* (*Arabidopsis thaliana* ozone induced gene 1; Sharma & Davis 1995)**





Genotypic variation in acidic phytohormone responses to ozone

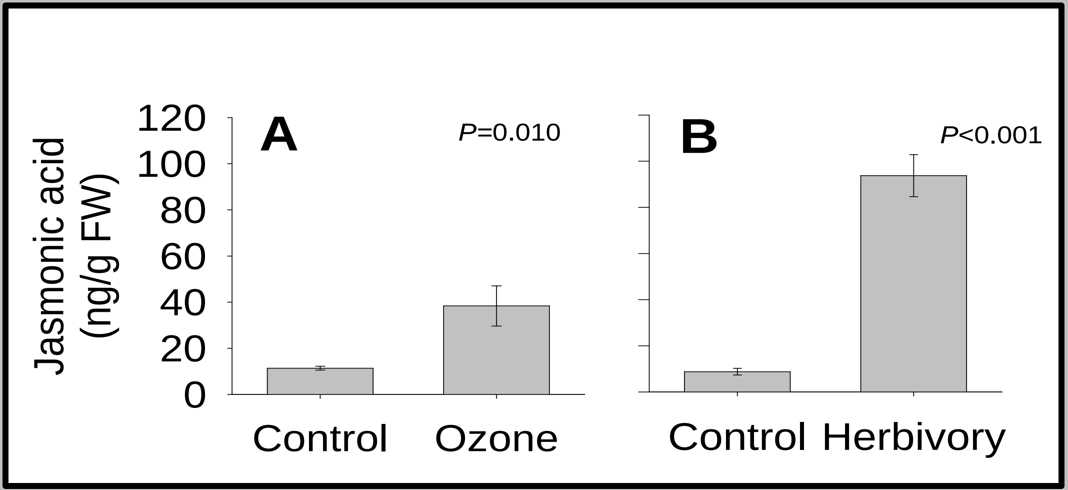


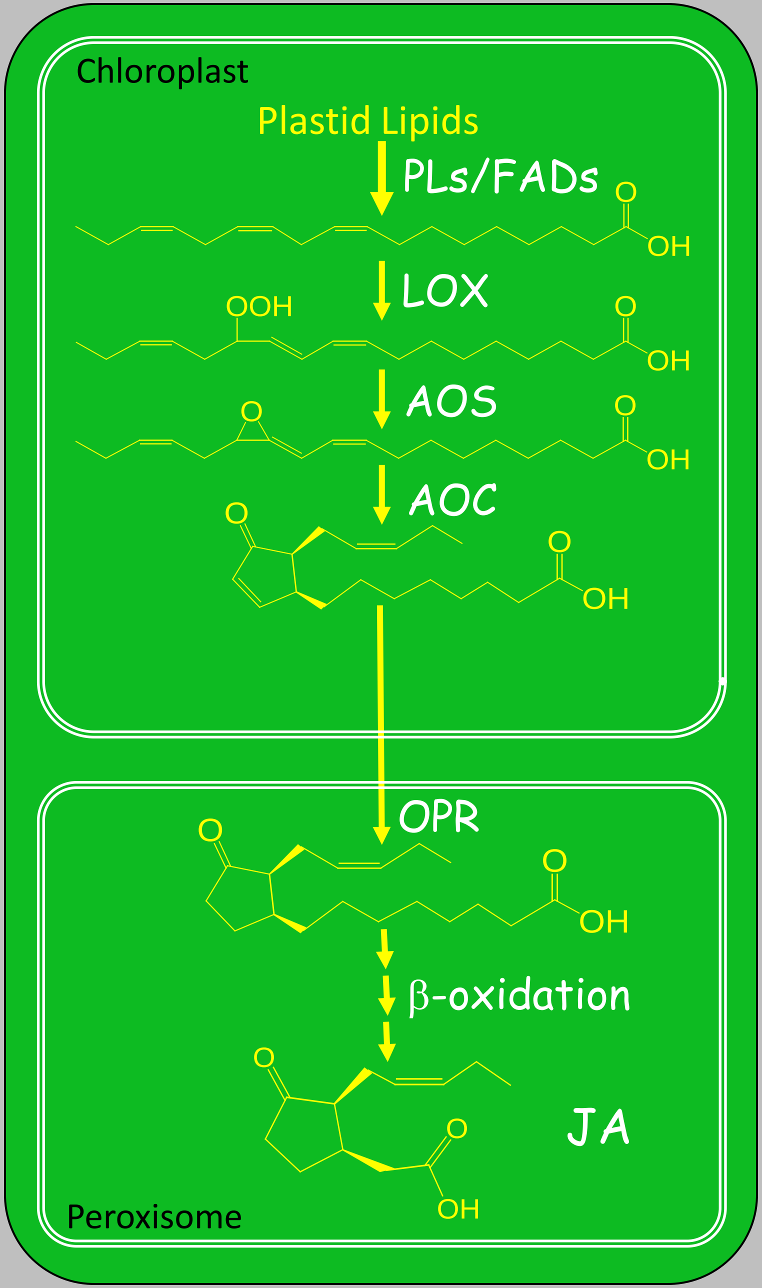
Orendovici-Best *et al.* (submitted)

Jasmonic Acid mediates induced resistance to herbivores

- JA pathway has been commonly used to measure a plant's ability to respond to both abiotic and biotic stimuli in *Arabidopsis*.

- JA mediates many plant defense responses, VOCs, secondary metabolites, other plant hormones, etc...





Interactions of Herbivory and Ozone?


Fumigation chambers


OGY

80 ppb O_3


Lymantria dispar herbivory

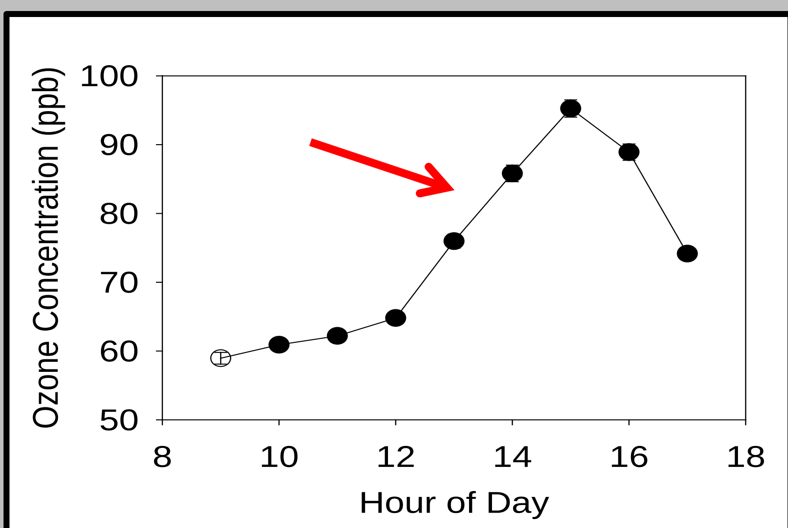
- Microarrays
- Metabolomics (1º and 2º)
- Functional Genomics
- Soil and Microbial Interactions

Experimental Design

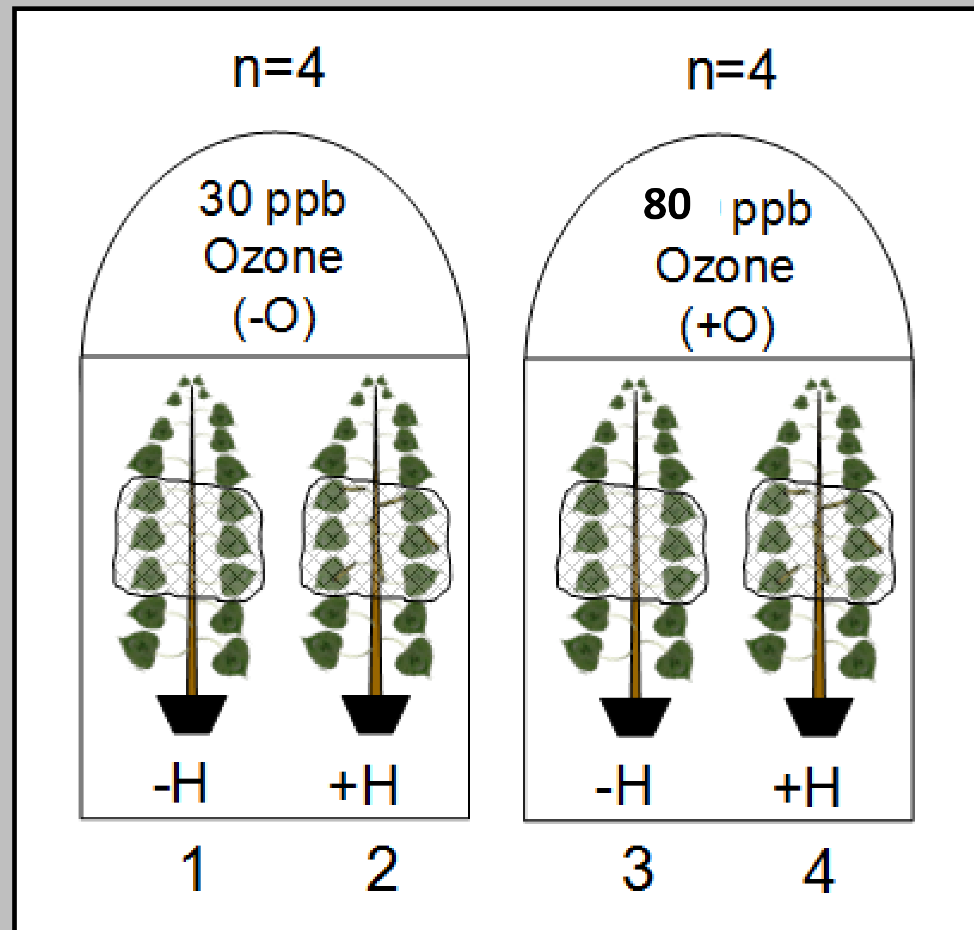
•Plants subjected to ozone fumigation for 1 week (control 30 ppb, treatment 80 ppb ozone).

• Sign of first symptoms, 24 hour with 10 gypsy moth caterpillars, then leaves collected

• RNA extracted, cDNA library constructed, NimbleGen Poplar array used.

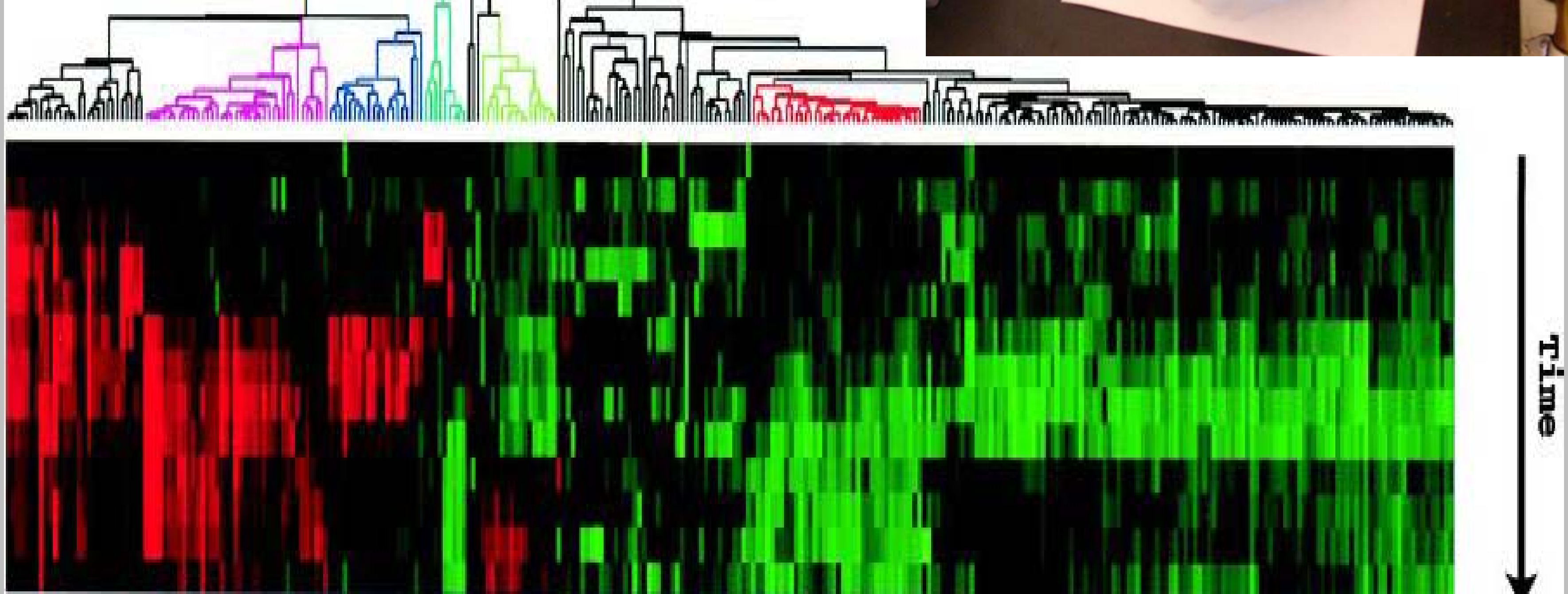


4 plants per fumigation chamber, 16 chambers.



Microarray Analysis of Ozone, Insect Herbivory, and Mycorrhizal Infection on *Populus*.

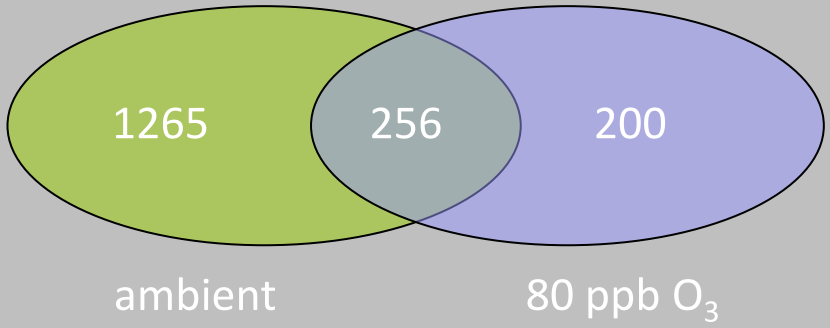
Abiotic and biotic stress responses, as well as mycorrhizal infection, modify plant gene expression.



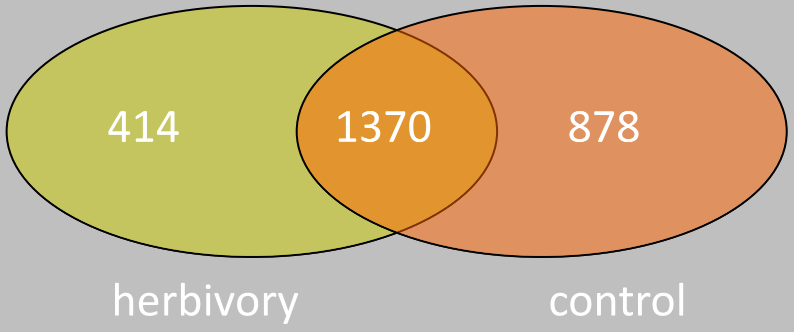
Ozone compromises induced responses to herbivores

Transcriptome changes compromised

Herbivory vs. Control



Ozone (80 ppb) vs. Ambient



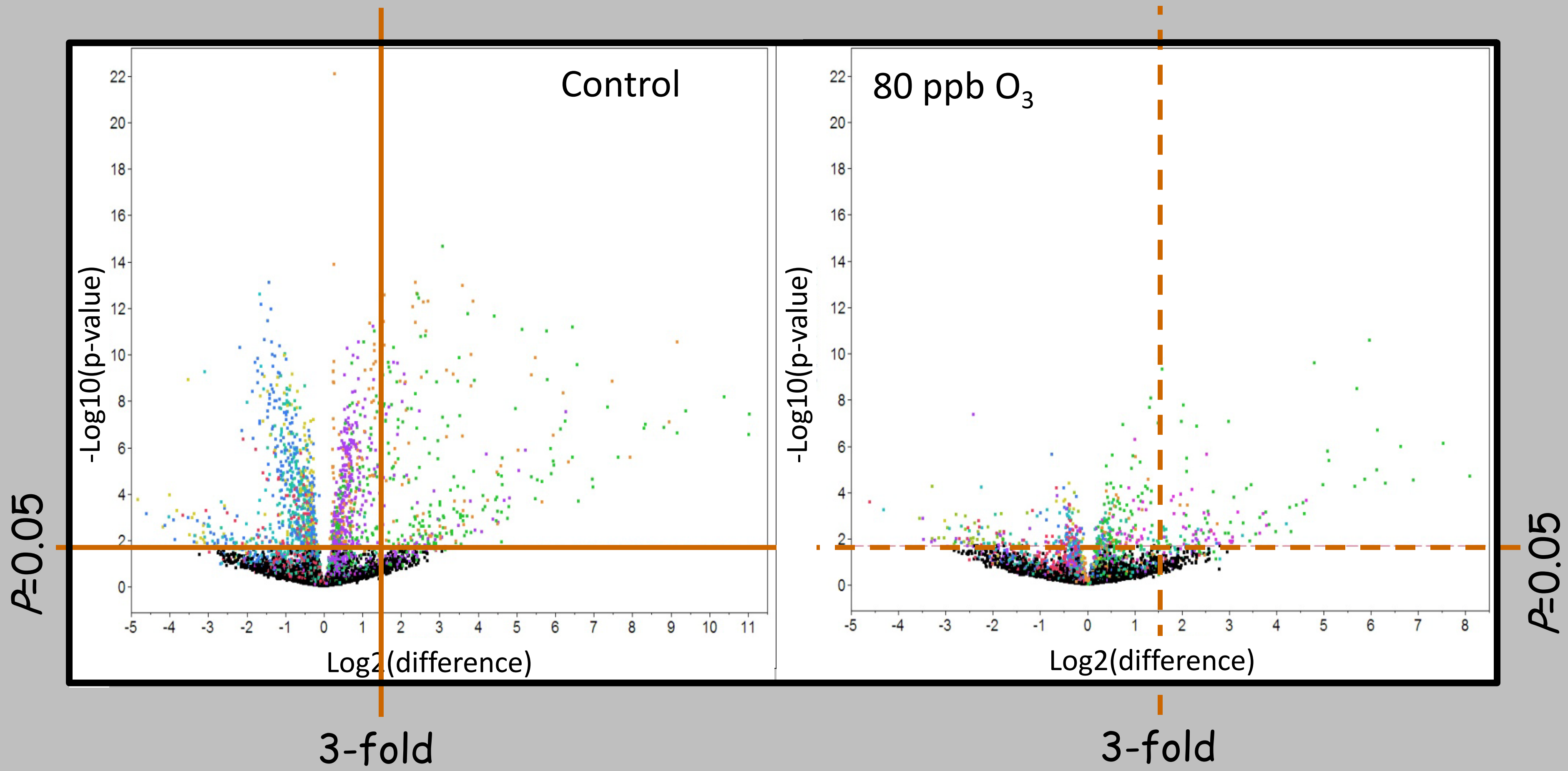
Herbivore-induced transcriptome influenced strongly by ozone

Ozone-induced transcriptome NOT influenced strongly by herbivory

Ozone compromises induced responses to herbivores

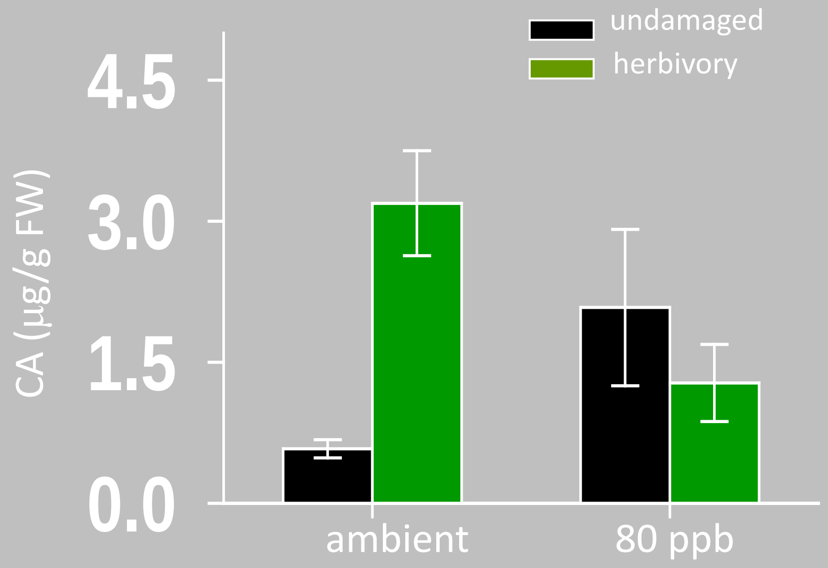
Transcriptome changes compromised

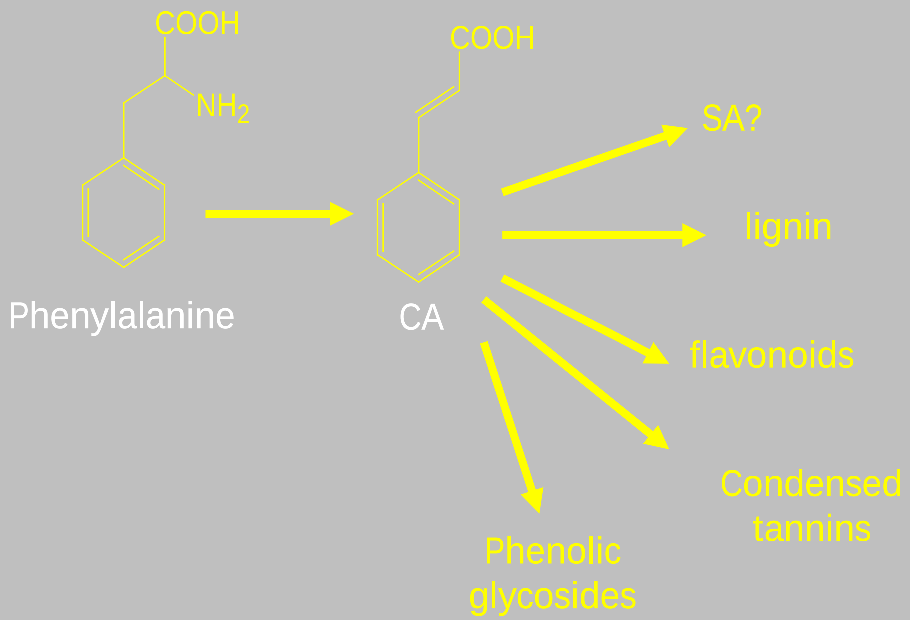
Herbivore treatment plants



Ozone mitigates herbivore-induced responses

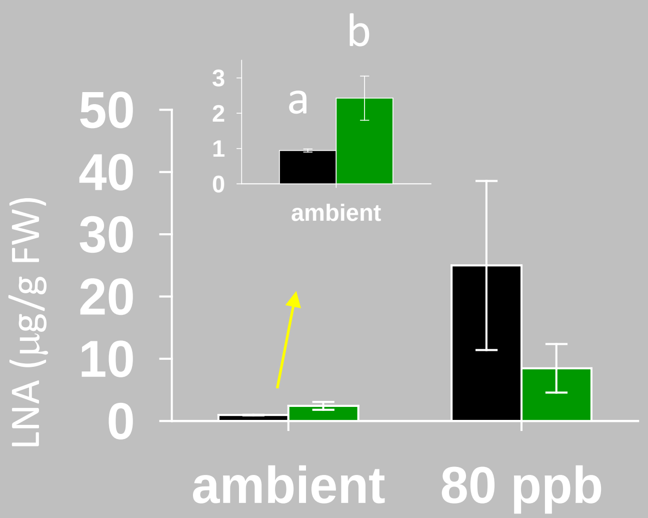
Phenylpropanoid pathway

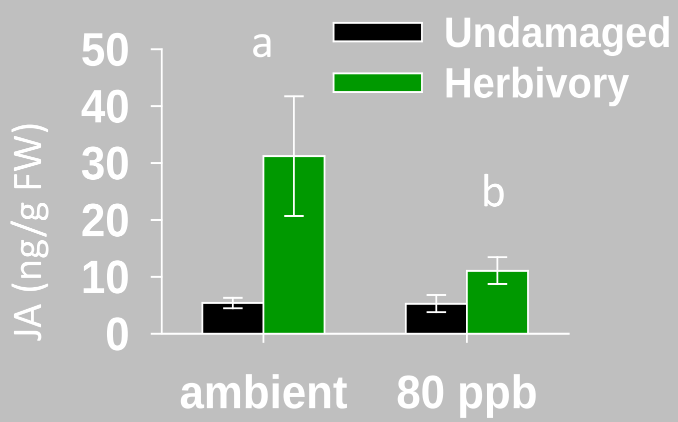


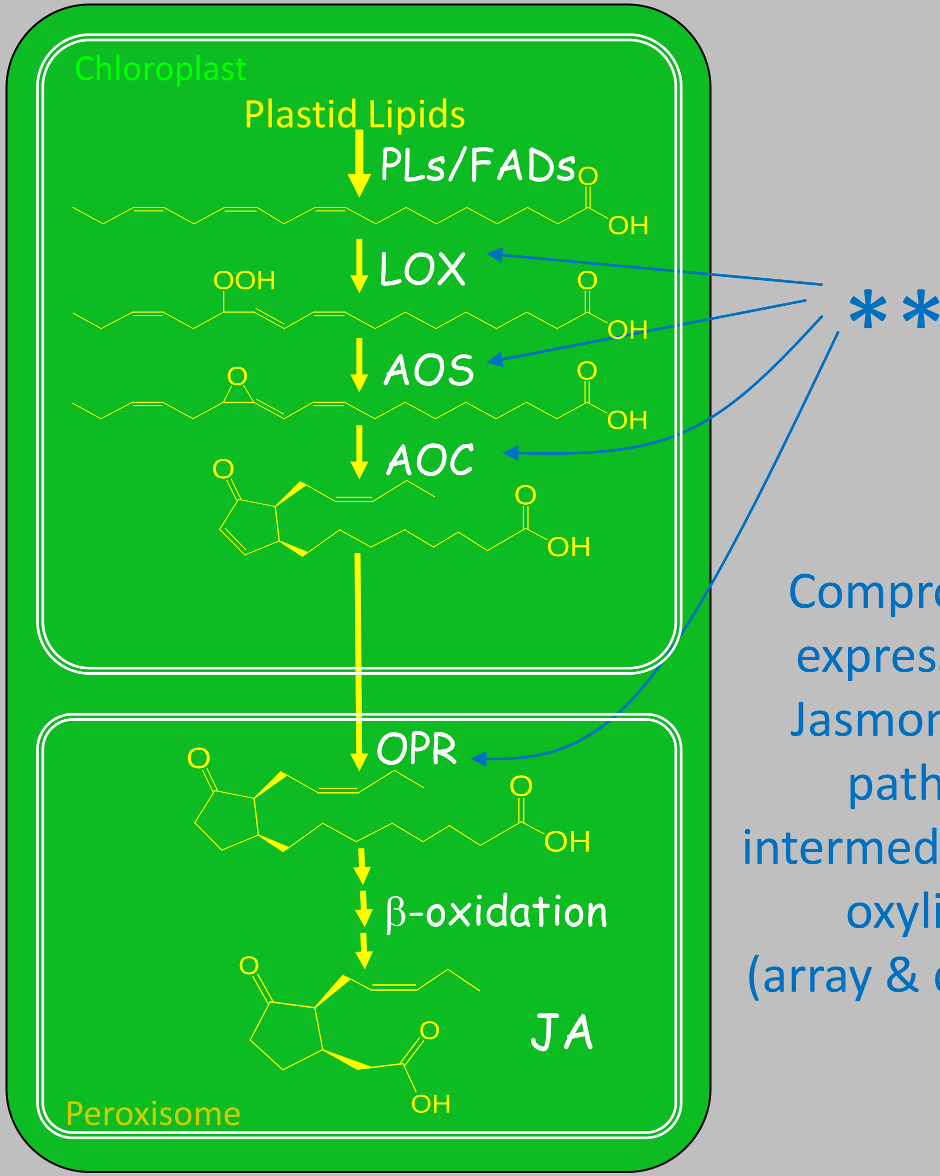


Ozone compromises induced responses to herbivores

Jasmonate synthesis

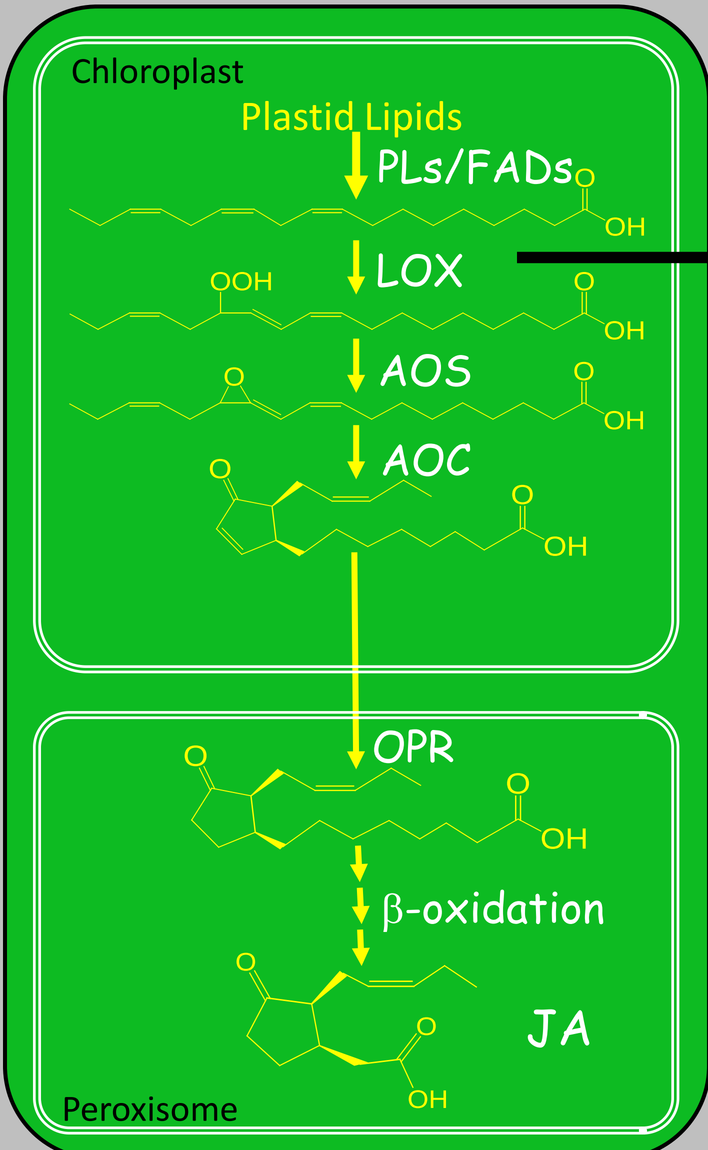






Compromised expression of Jasmonic acid pathway intermediates and oxylipins (array & qRT-PCR)

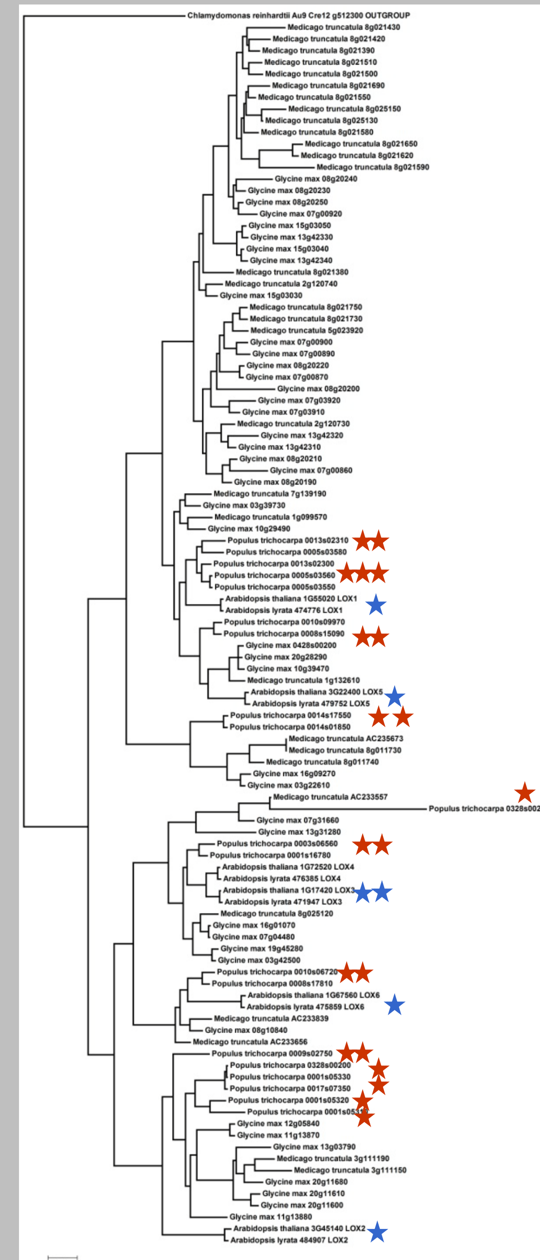
Molecular Phylogenies of JA pathway genes in *Populus*



LOX: At=6; Pt=20

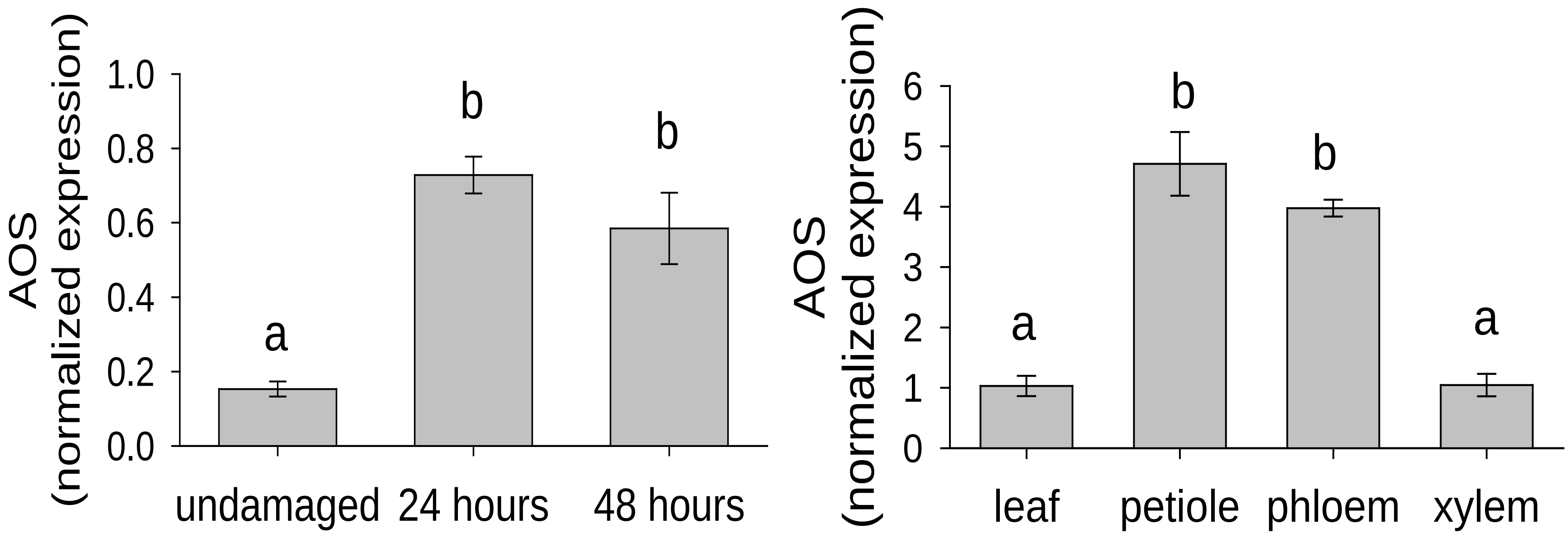
Populus
Arabidopsis

Herr et al. in prep



- Phylogeny shown constructed with genes from *Arabidopsis thaliana*, *Populus trichocarpa*, *Glycine max*, and *Medicago truncatula*.
- Over 400 genes for plants with sequenced genomes.
- Large "LOX family" contains α-DOXs, Lipases, and large numbers of LOX genes.

Gene expression data (qRT-PCR) for AOS in specific Poplar tissue types. Gene expression studies were carried out in hybrid poplar (OGY: *P. deltoides* x *P. nigra*).

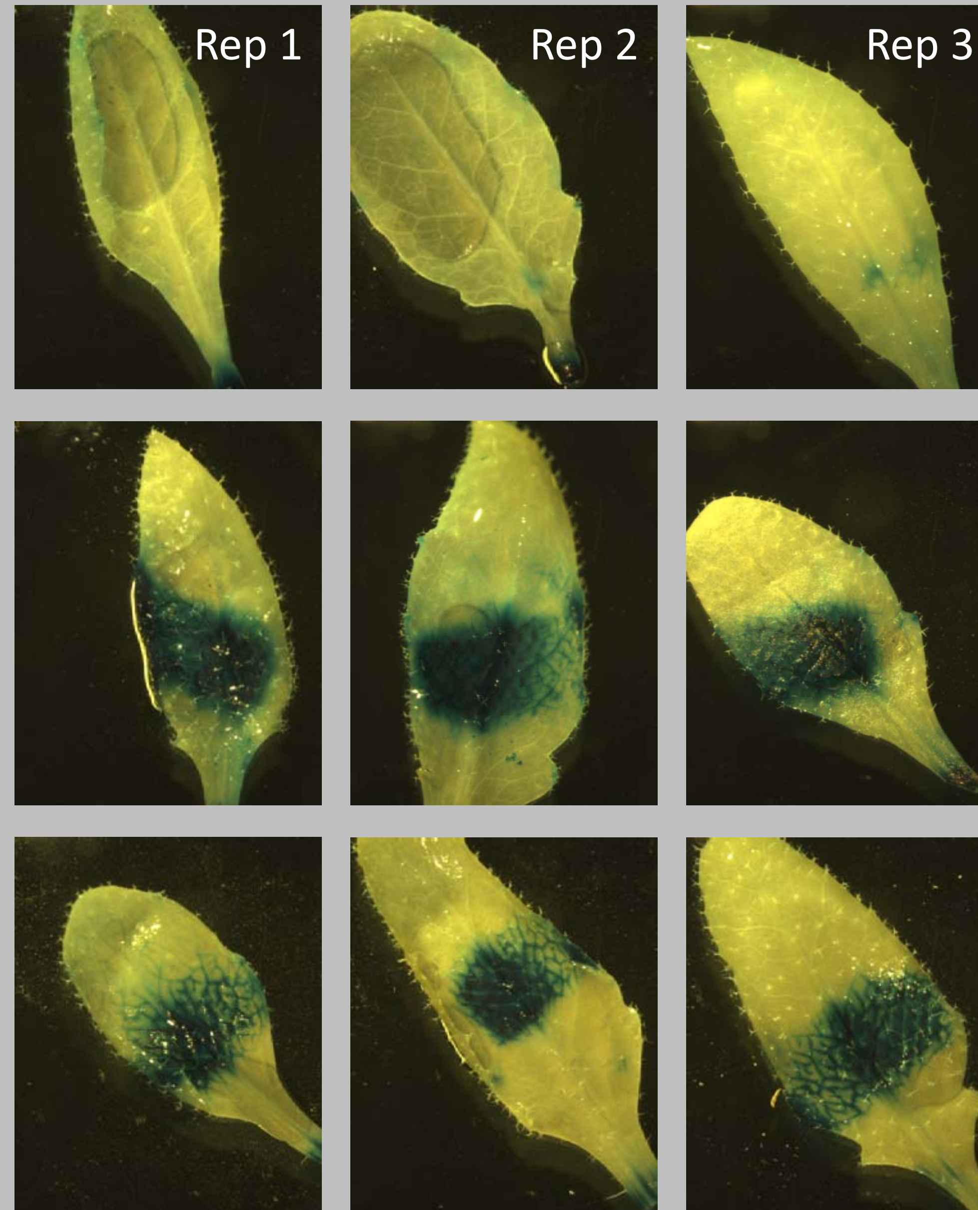


GUS-*AOS* reporter assay - 0 hours after wounding (collected immediately before fixing)

Unwounded companion

Stained immediately

Fixed after 1 minute

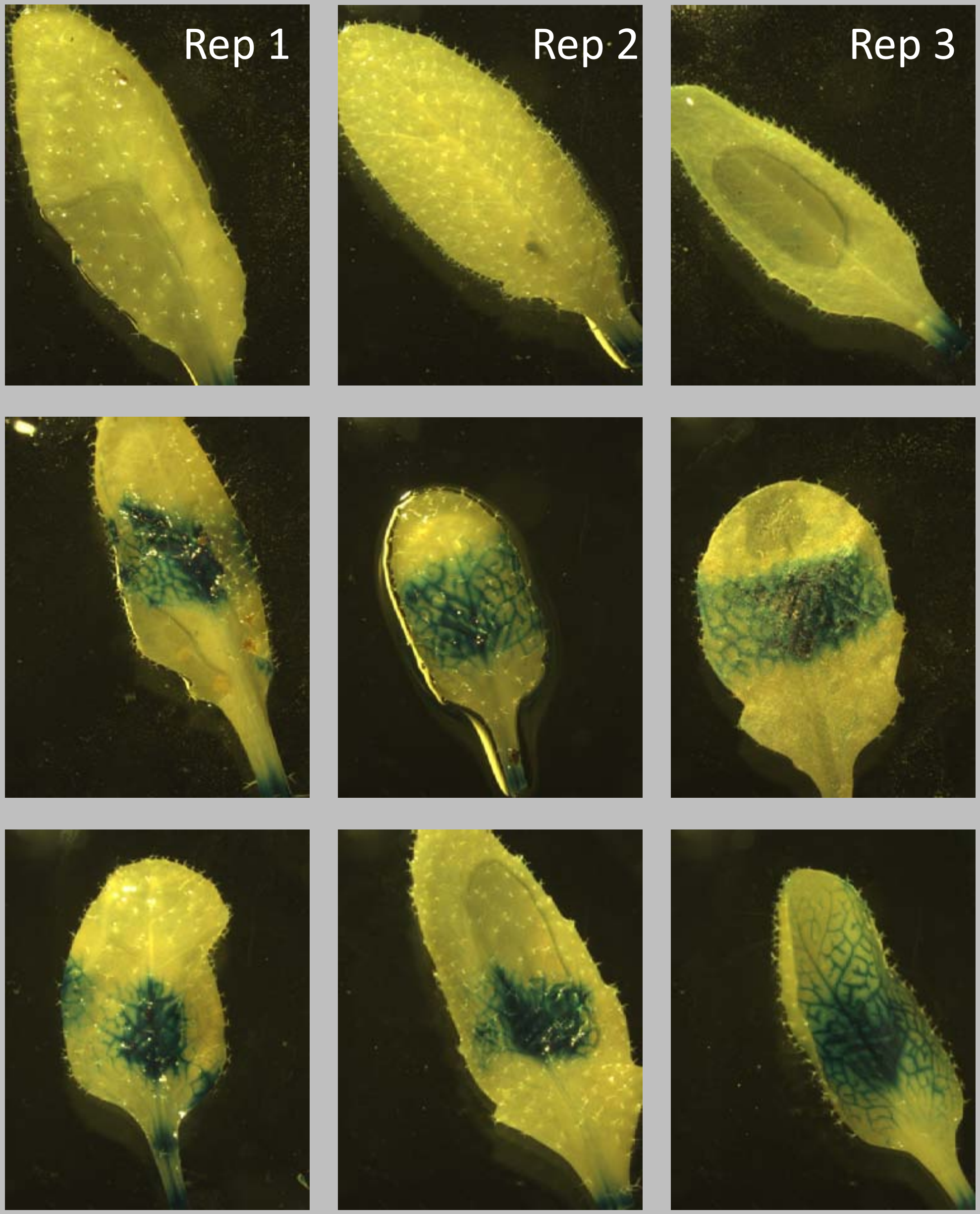


GUS-*AOS* reporter assay – 1 to 2 hours after wounding (collected immediately before fixing)

Unwounded companion

Fixed after 1 hour

Fixed after 2 hours

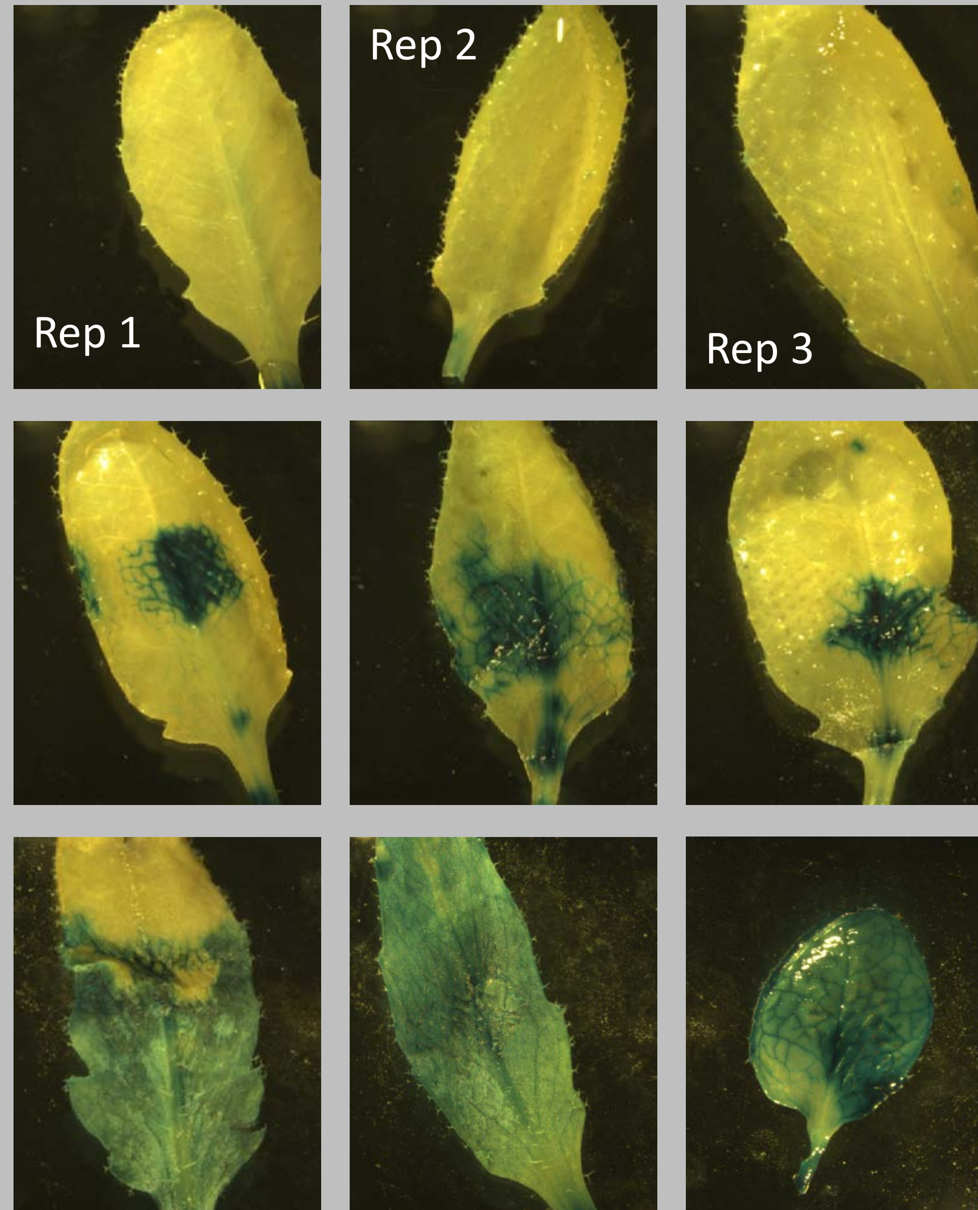


GUS-*AOS* reporter assay – 6 to 24 hours after wounding (collected immediately before fixing)

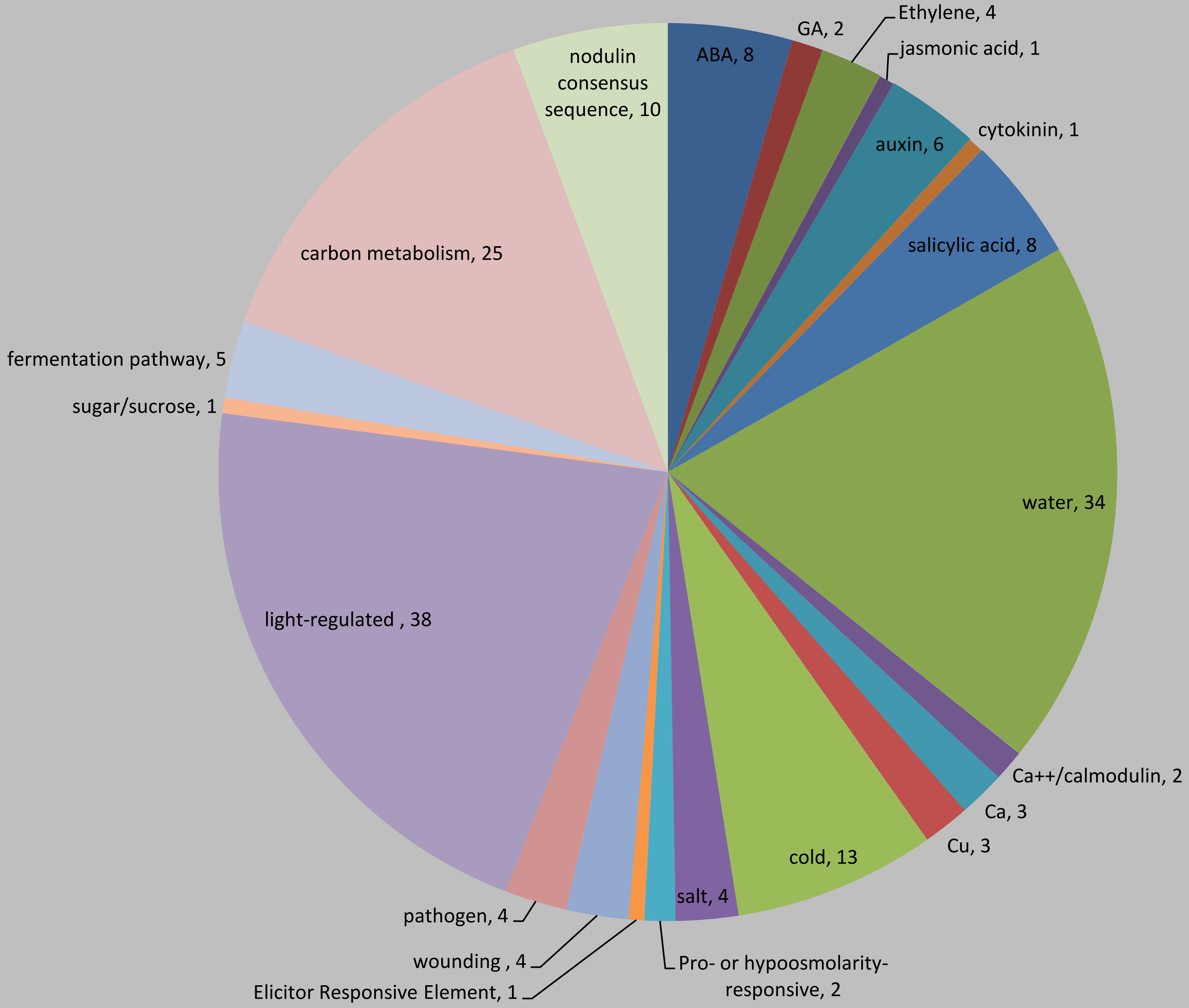
Unwounded companion

Fixed after 6 hours

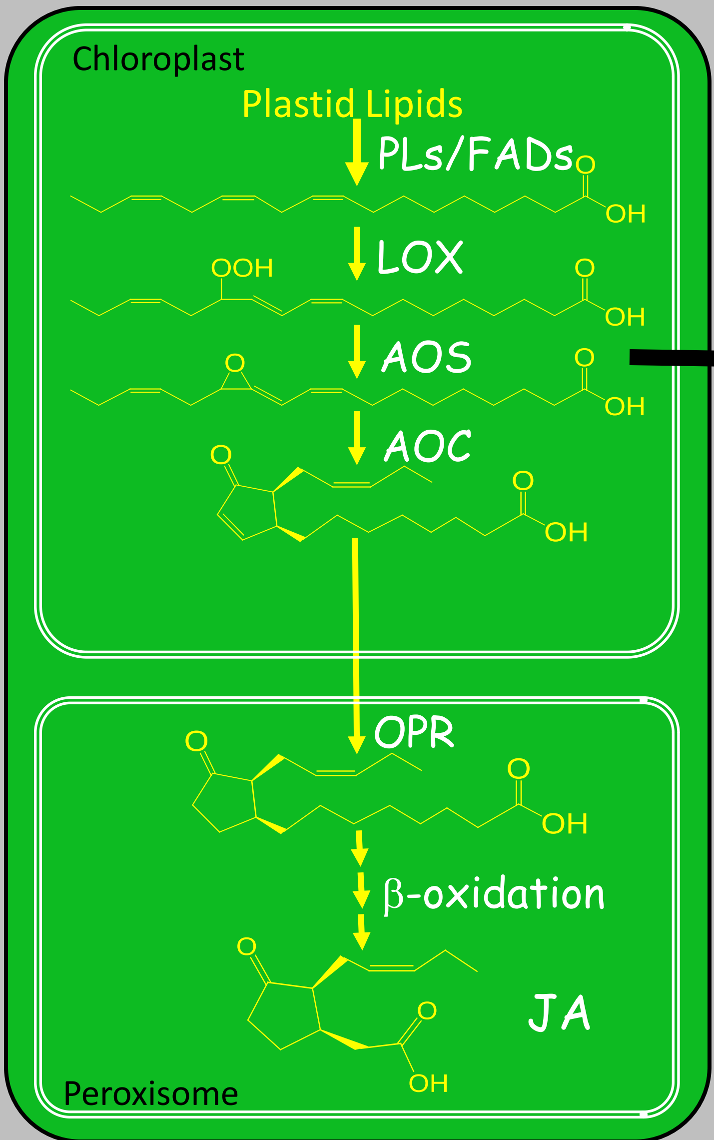
Fixed after 24 hours



Populus AOS promoter elements



Molecular Phylogenies of JA pathway genes in *Populus*



LOX: At=6; Pt=20

AOS: At=1; Pt=2

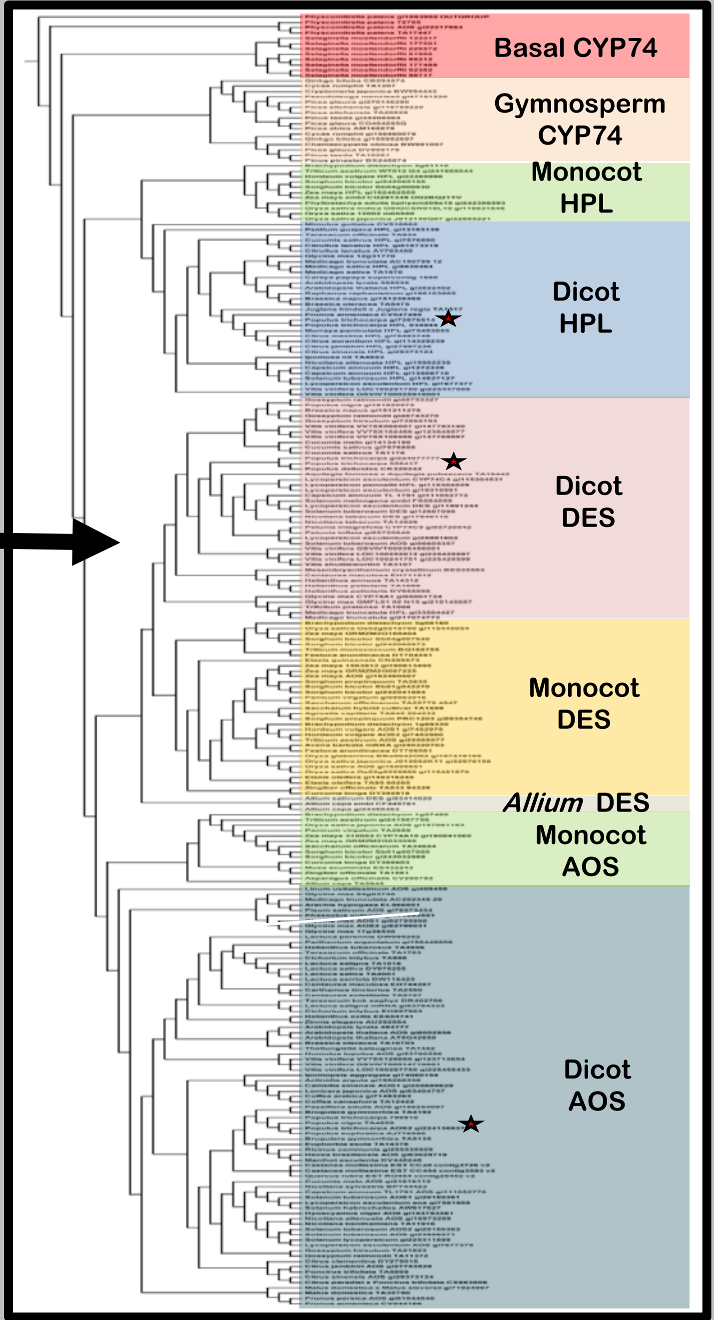
HPL: At=1; Pt=1

DES: At=0; Pt=3

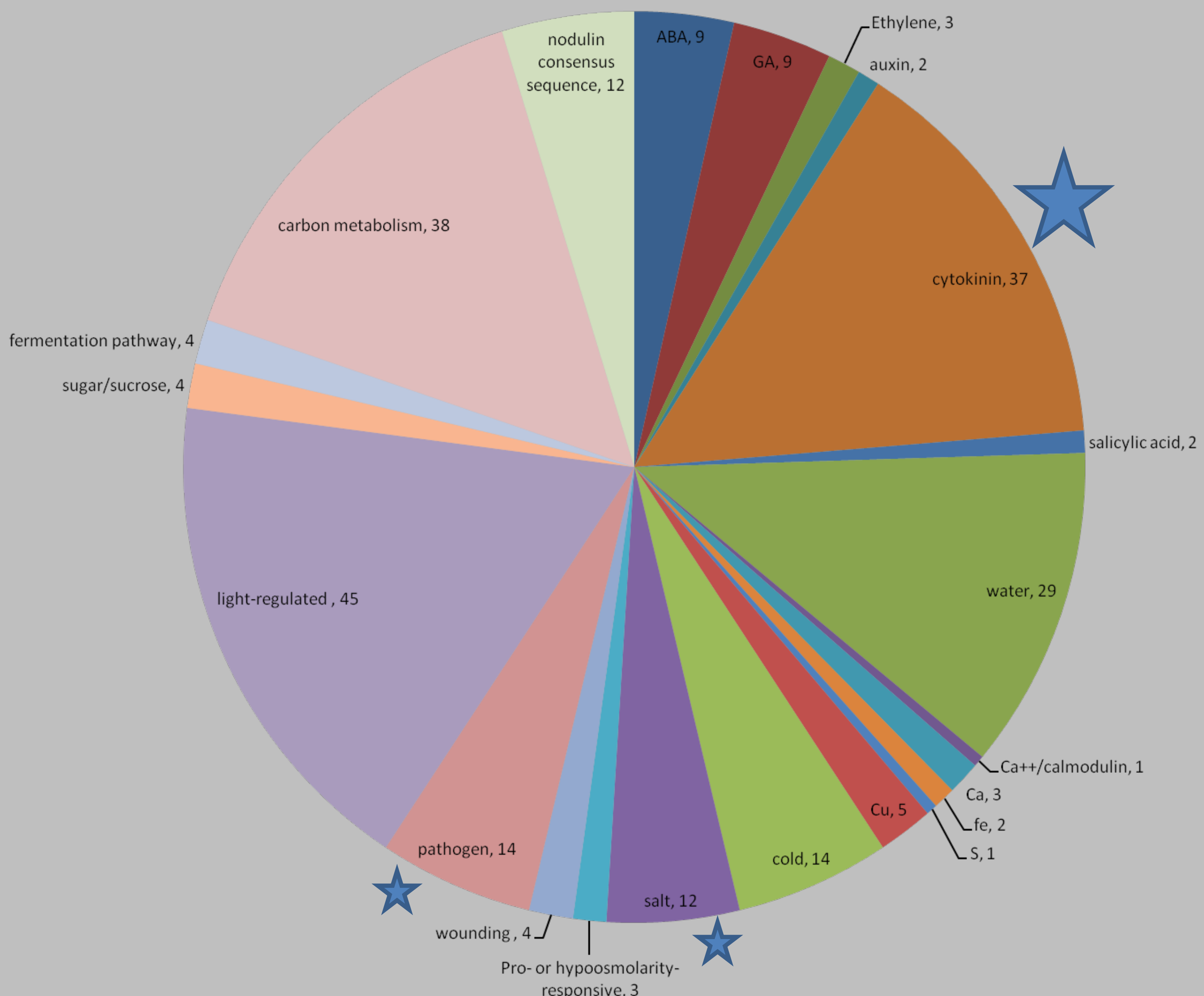
• Still more work needs to be done to understand oxylipin diversity, especially in *Populus*.

• Current focus is the elicudation of tissue specific expression in *Populus*.

Herr et al. in prep



Populus HPL promoter elements



Synopsis for the Primary Step in Oxylipin Synthesis

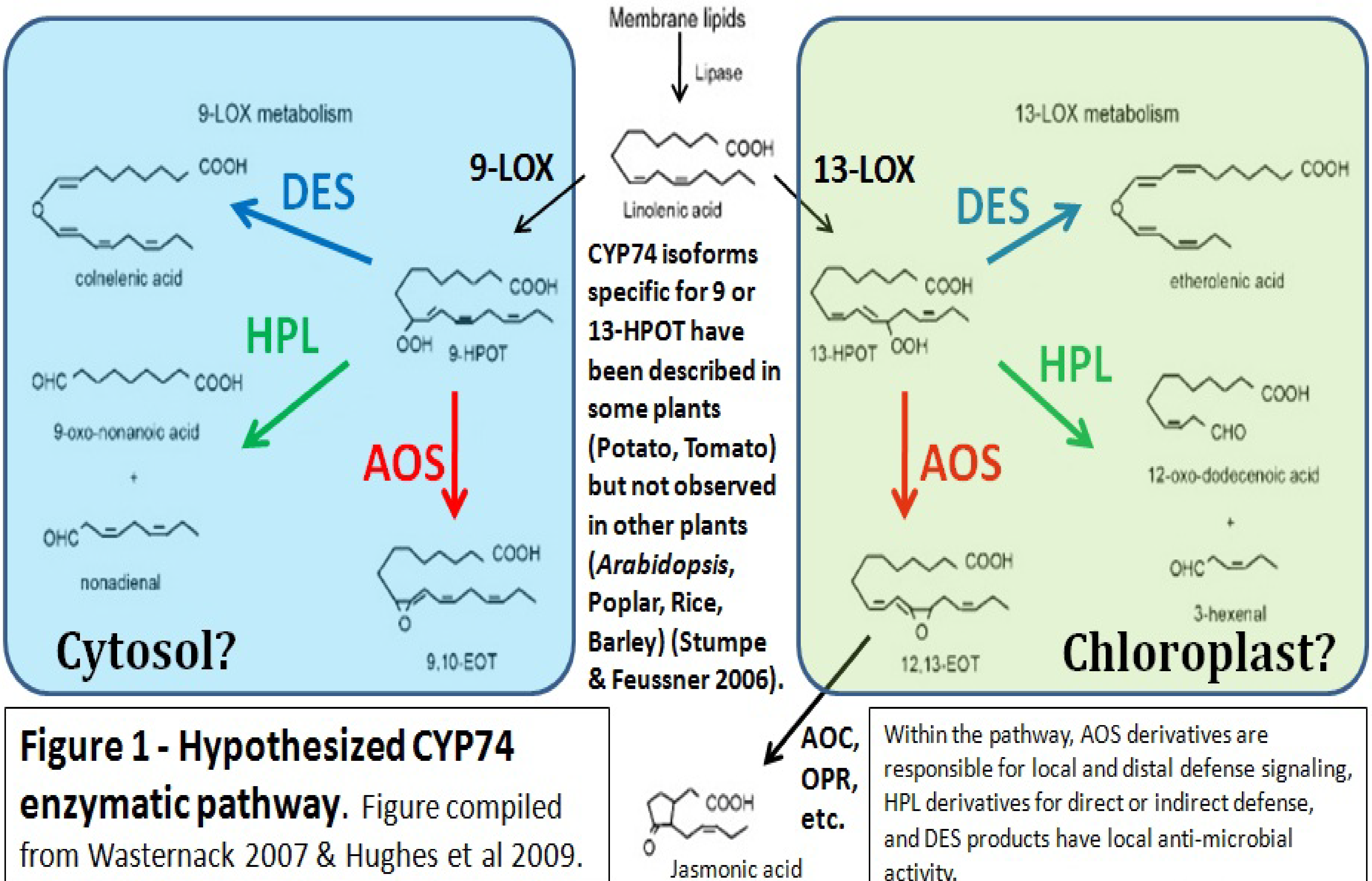


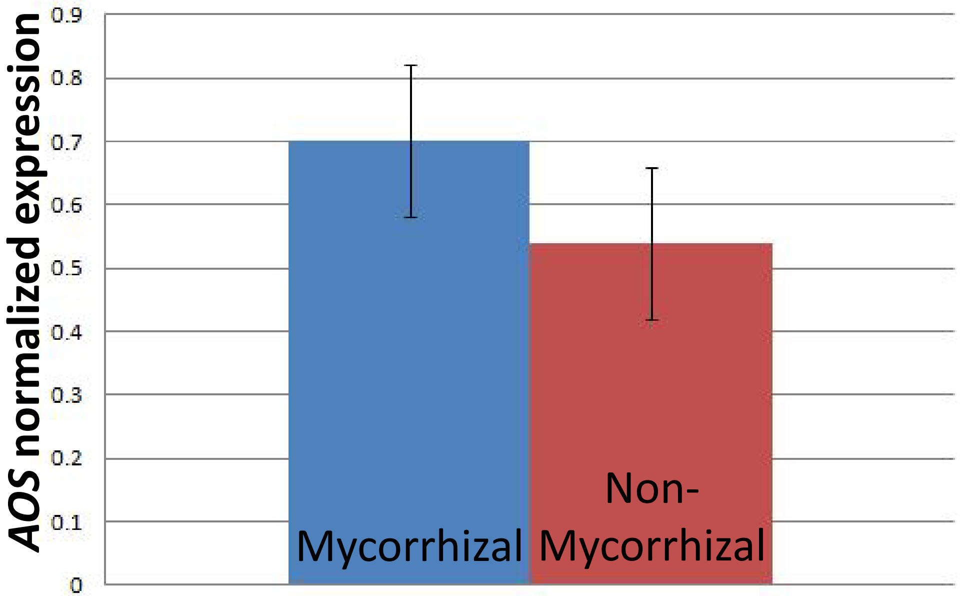
Figure 1 - Hypothesized CYP74 enzymatic pathway. Figure compiled from Wasternack 2007 & Hughes et al 2009.

Within the pathway, AOS derivatives are responsible for local and distal defense signaling, HPL derivatives for direct or indirect defense, and DES products have local anti-microbial activity.

Role of Ectomycorrhizae in Poplar Ozone fumigation.

- Mycorrhizal (*Laccaria bicolor*) and Non-Mycorrhizal poplar were established and mycorrhizal root tips were counted in all of the plants after the experiment (70% root infection in mycorrhizal plants)

Relative *AOS* expression in mycorrhizal & non-mycorrhizal plants

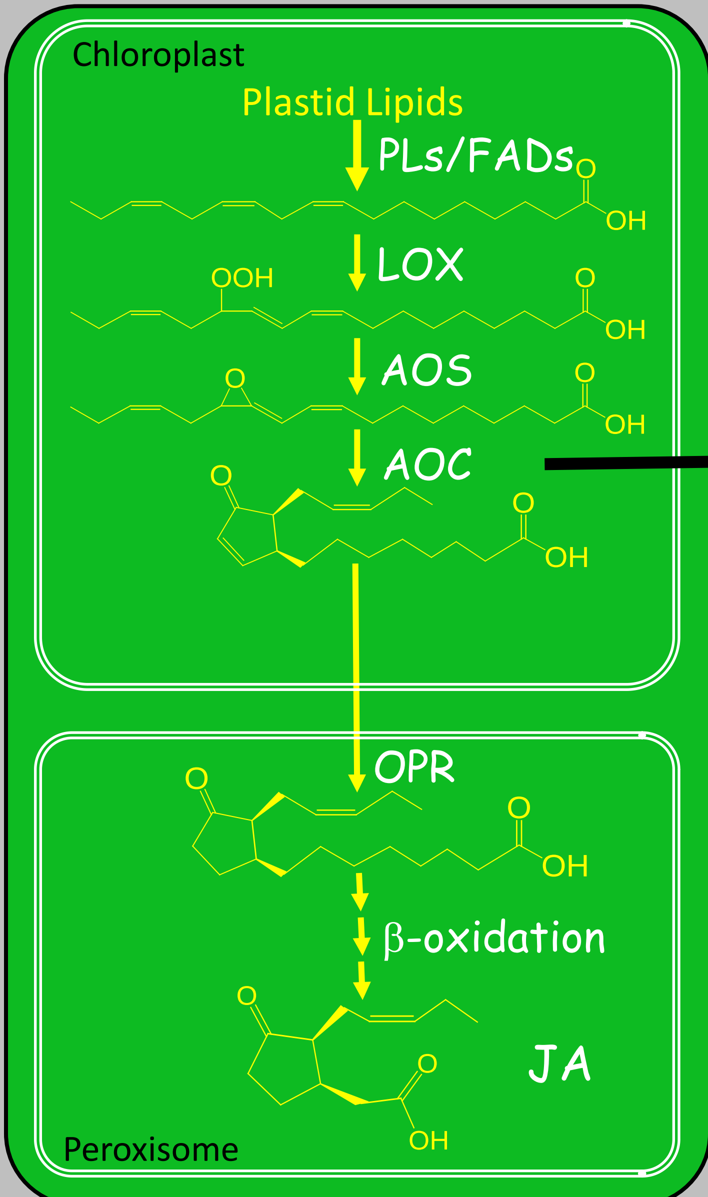


- There are 3 orthologs of the CYP74 family in *Laccaria*. How volatiles from *Laccaria* affect poplar gene expression is unknown.

- Orthologs exist in many other fungi and bacteria (Keller et al 2009).

- We know that Ozone increases the volatility of plant isoprenes, products from CYP74s are very similar (Loreto et al 2001, Sharkey et al 2008).

Molecular Phylogenies of JA pathway genes in *Populus*

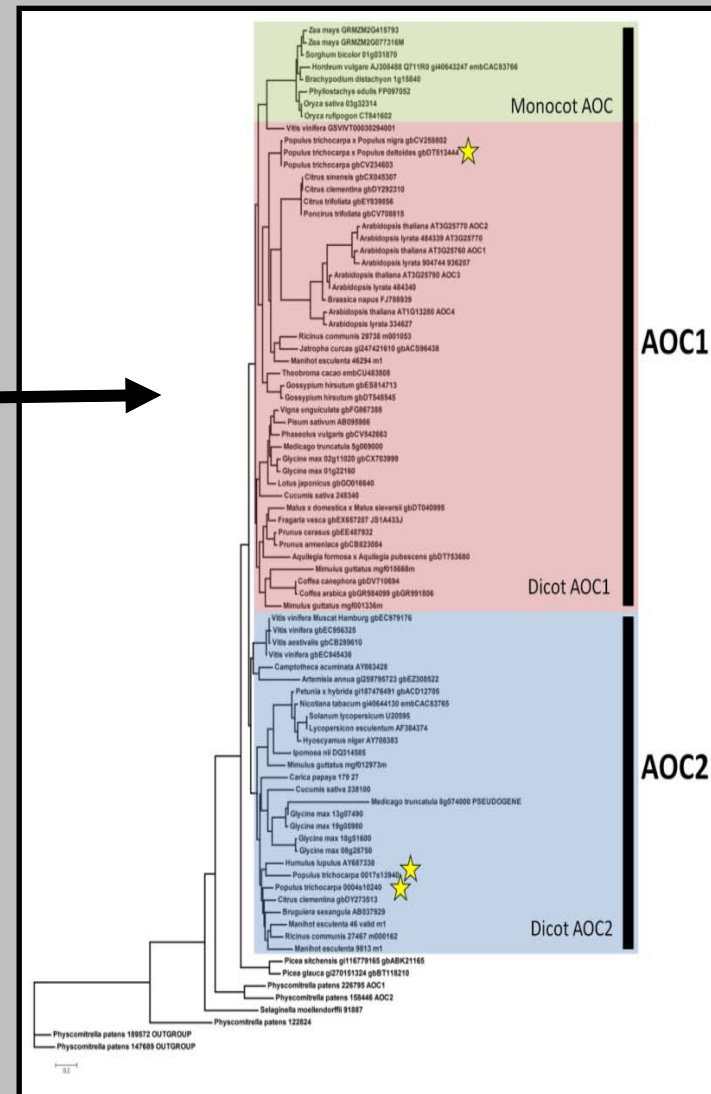


LOX: At=6; Pt=20

AOS: At=1; Pt=2

AOC: At=4*; Pt=3

* Tandem duplication



- AOC1 genes involved in JA pathway for each *Arabidopsis* and *Populus*. No AOC2 in *Arabidopsis*.

- AOC2 in *Populus* appears to be expressed during root and pollen development.

Herr et al. in prep

Immediate Research Directions

- Insect growth quantification on both ozone fumigated and non-fumigated *Populus* (Reviewer Requested).
- Microarray Run or Re-trial of *Laccaria-Populus* Ozone experiment?
- Further Characterization of the Jasmonic Acid pathway in *Populus* (Reviewer Requested, qRT-PCR data validation)
- Below-ground community structure across high vs. low ozone sites?

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- Understanding Stress Responses in Hybrid Poplar Biomass Plots
    - Tree Spacing, Growth and the Use of 1-MCP (Ethylene Blocking Agent) in Stress Response
    - Soil Metagenomics of Poplar Biomass Plantations
- Final Conclusions


OfficeMax

Hybrid Poplar Biomass Field Study

- Study Consists of Test of Tree Spacing, Treatment of Ethylene Blocking Agent 1-MCP, and Presence of Nitrogen Fixing Biomass Tree (Black Locust, *Robinia*) on Poplar Biomass Production
- Thirty Plots Covering Two Sections of Marginal Farm Land (15 each for Spacing only / Black Locust and Spacing). Half of the Plots From Each Section Receive 1-MCP
- Measurements Include: Biomass Production (Ethanol Conversion), Role of 1-MCP in Digestibility and Stress Mediation, Plant Growth Characteristics, Soil Microbe Composition and Change


Plot installation, May 2009

July 22nd

1-MCP spraying,
June & August

October 14th: leaf metrics,
height measurements

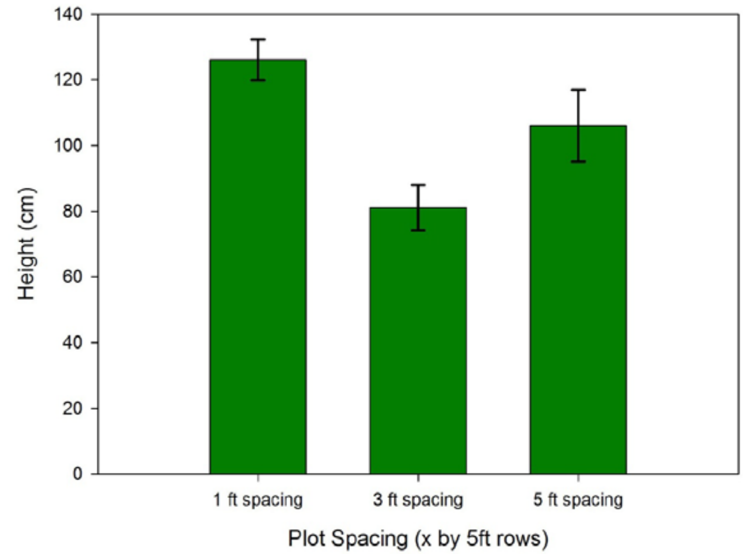

Figure 1 – Average tree height at three plant spacing regimes.

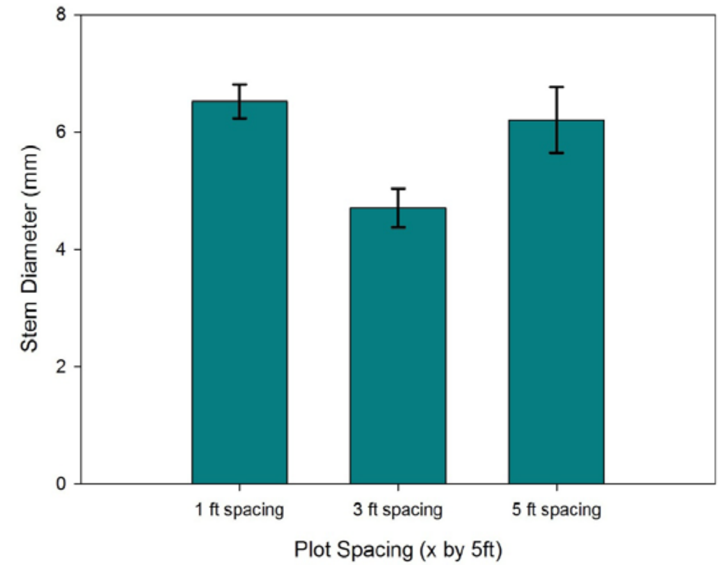

Figure 2 – Average stem diameter at three plant spacing regimes.

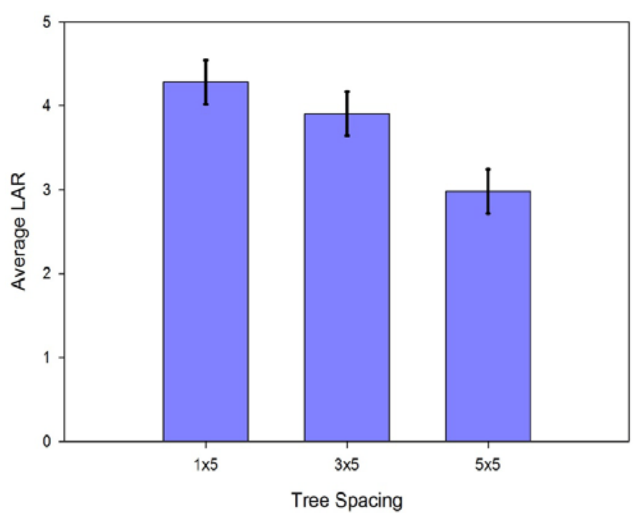

Figure 3 – Average LAR (leaf area removed) from herbivory at three plant spacing regimes.

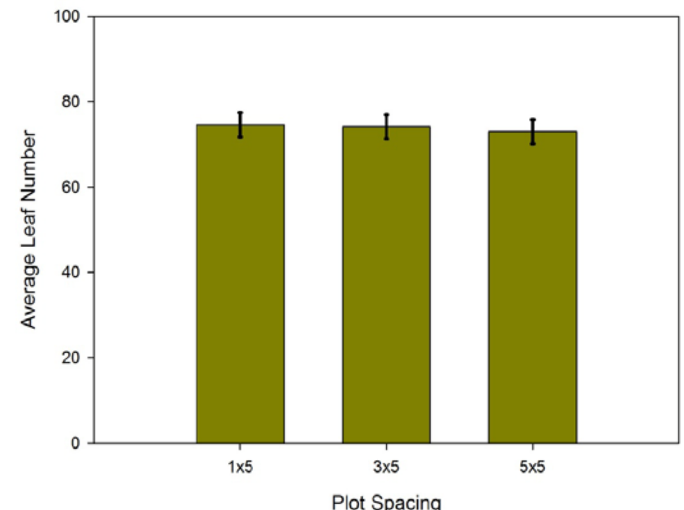

Figure 4 – Average number of leaves per tree at three plant spacing regimes.

Future Research Directions for Biomass Plantations

- Growth characteristics in relation to biomass production.
- Growth characteristics and changes in plant phenology in biomass plots with/without 1-MCP.
- Below-ground community structure within tree spacing and 1-MCP treated plots.
- Impact of plant succession on below-ground microbial components and vice versa.
- Insect diversity and leaf area damage.


30 year old hybrid poplar plantation

Photo: Marc Buee

Metagenomics of Forest Soils in Brueil

- Expansion of the Buee *et al* 2009 New Phytologist) test study.
- Sampling in six forest types (five 30-year old monoculture plots, One “undisturbed plot”)
- Each plot has 30 years of geochemical data and 10 years of soil respiration measurements to accompany our sampling
- Six random samples from center of plots
- Six types include: Mixed Hardwoods (“Undisturbed” site: mainly Beech & Oaks), Douglas Fir (*Pseudotsuga*), Norman Fir (*Abies*), Oak (*Quercus*), Beech (*Fagus*), and Pine (*Pinus*)
- Sampling done seasonally (spring & autumn)


Marc Buee

Future Research Directions

- Metagenomics of Ectomycorrhizal Communities.
- Gene expression of “key” gene in forest and biomass plantation soils.
- Comparative genomics of Fungi and Plants (Ectomycorrhizal Fungi in particular).
- Symbiotic associations of host specificity in Ectomycorrhizal Symbioses.

Acknowledgements

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The Schatz Center for Tree Molecular Genetics

NSF National Science Foundation WHERE DISCOVERIES BEGIN

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School of Forest Resources

Thank you!

Merci Beaucoup!