Small Chemicals with Big Impact:

Investigating Terpenoid Metabolism

from Plant-Environment Interactions to Bioproducts

Philipp Zerbe

UC DAVIS
COLLEGE OF BIOLOGICAL SCIENCES

https://zerbelab.weebly.com/

Plants employ a vast repertoire of metabolites to mediate developmental processes and interactions with their environment, directly impacting survival and vigor.

Pollinator Interactions

Growth & Development

Microbiome Interactions

Pest & Pathogen Defenses

Abiotic Stress Tolerance

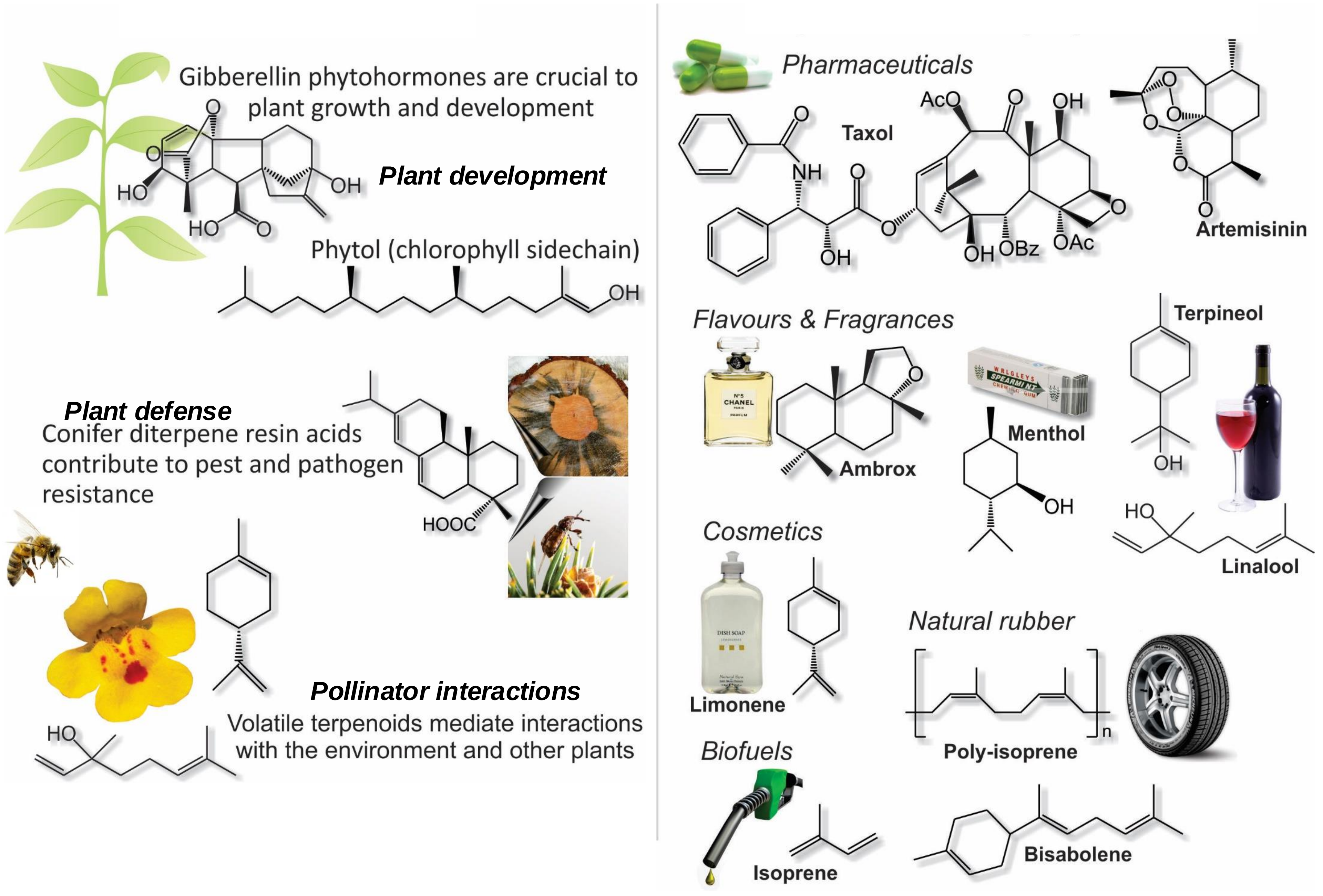
Allelopathy

UC DAVIS
UNIVERSITY OF CALIFORNIA

Understanding Plant Metabolism is critical for addressing global challenges of climate extremes, agriculture, food safety, bioenergy and healthcare.

> 70% of global calories are provided by few major crops (corn, rice, wheat)
~ 9 Metric tons of biofuel annually (maize, sorghum, switchgrass)
~ 25% of WHO medicines are plant-based
- Many other industrial bioproducts

Terpenoids have critical biological functions and many industrial applications



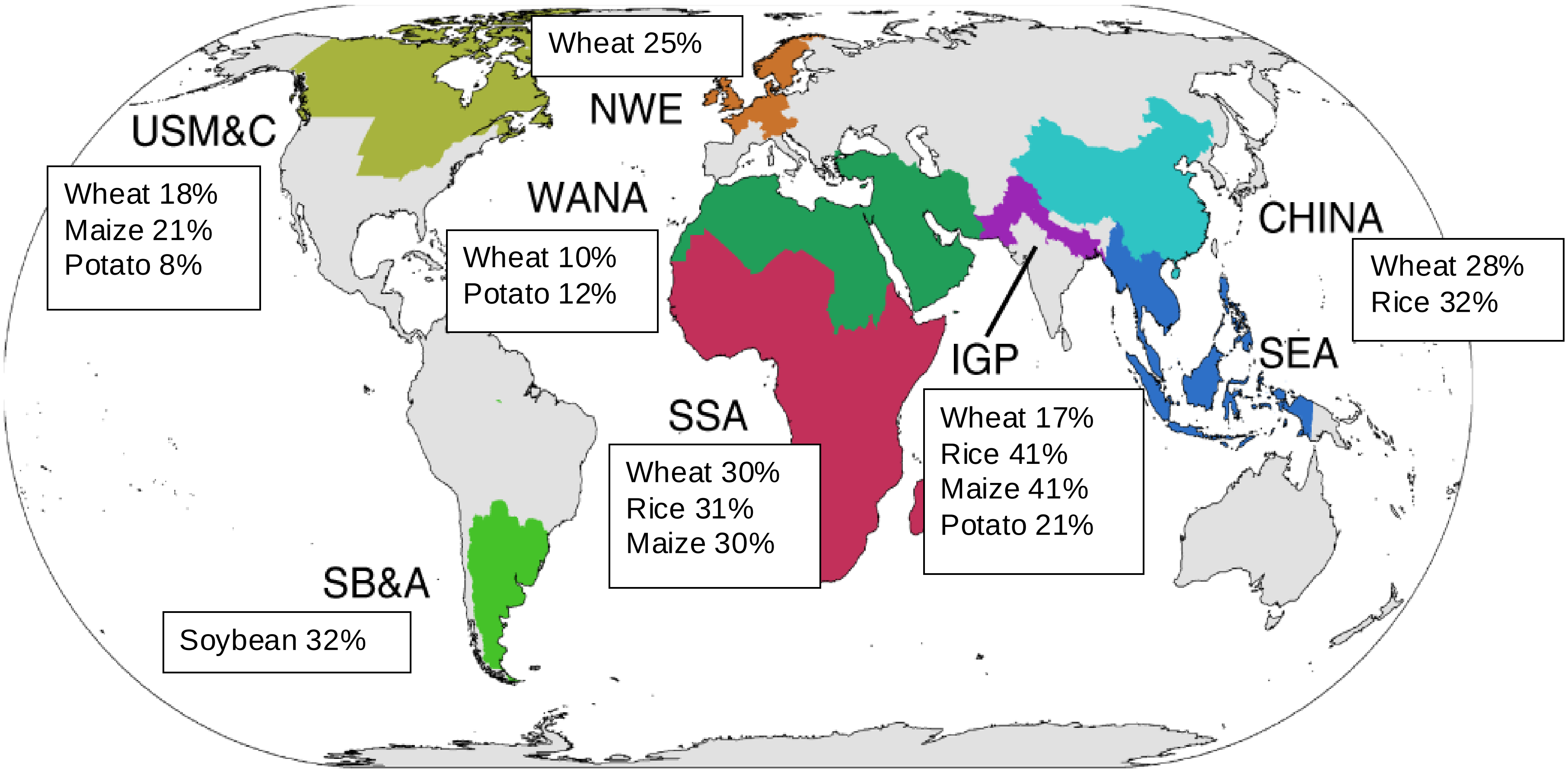
Knowledge of the biosynthesis and function of plant terpenoid metabolites in plant growth and environmental adaptation can aid the development of new strategies for crop improvement and bioproduct engineering

Terpenoid-mediated stress resilience in food and bioenergy crops

Defining terpenoid aroma metabolism for crop breeding

Enzyme discovery and engineering toward terpenoid bioproducts

Combined climate, pest & pathogen pressures increase crop losses



Adapted from Savary *et al*. 2019, *Nat Ecol Evol* 3:430

UC DAVIS
UNIVERSITY OF CALIFORNIA

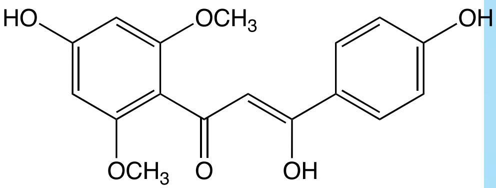
Estimated 2023 US maize yield losses due to diseases & mycotoxin contamination

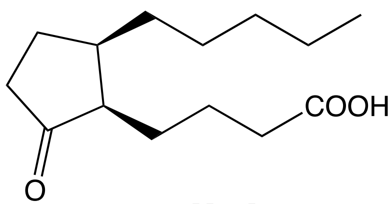
Disease	Total US losses (millions of bushels)
Root Rots and Seedling Blights	
Nematodes[1]	**56.4**
Root rots	1.4
Leaf and Aboveground Diseases	
Tar spot	38.1
Gray leaf spot	18.6
Northern corn leaf blight	7.3
Bacterial leaf streak	5.8
Stalk Rots	
Fusarium stalk rot	**49.5**
Anthracnose stalk rot	**47.1**
Diplodia stalk rot	34.0
Gibberella stalk rot	26.4
Crown rot	23.4
Charcoal rot	23.1
Ear Rots	
Gibberella ear rot	**49.4**
Fusarium ear rot	**46.9**
Diplodia ear rot	10.0

Source: Crop Protection Network

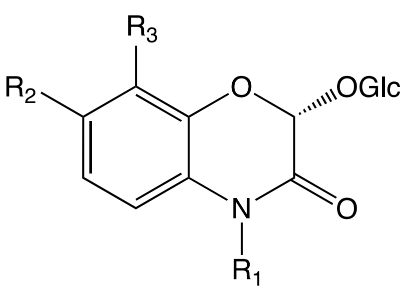
Maize produces a complex blend of chemical defense compounds

Flavonoids

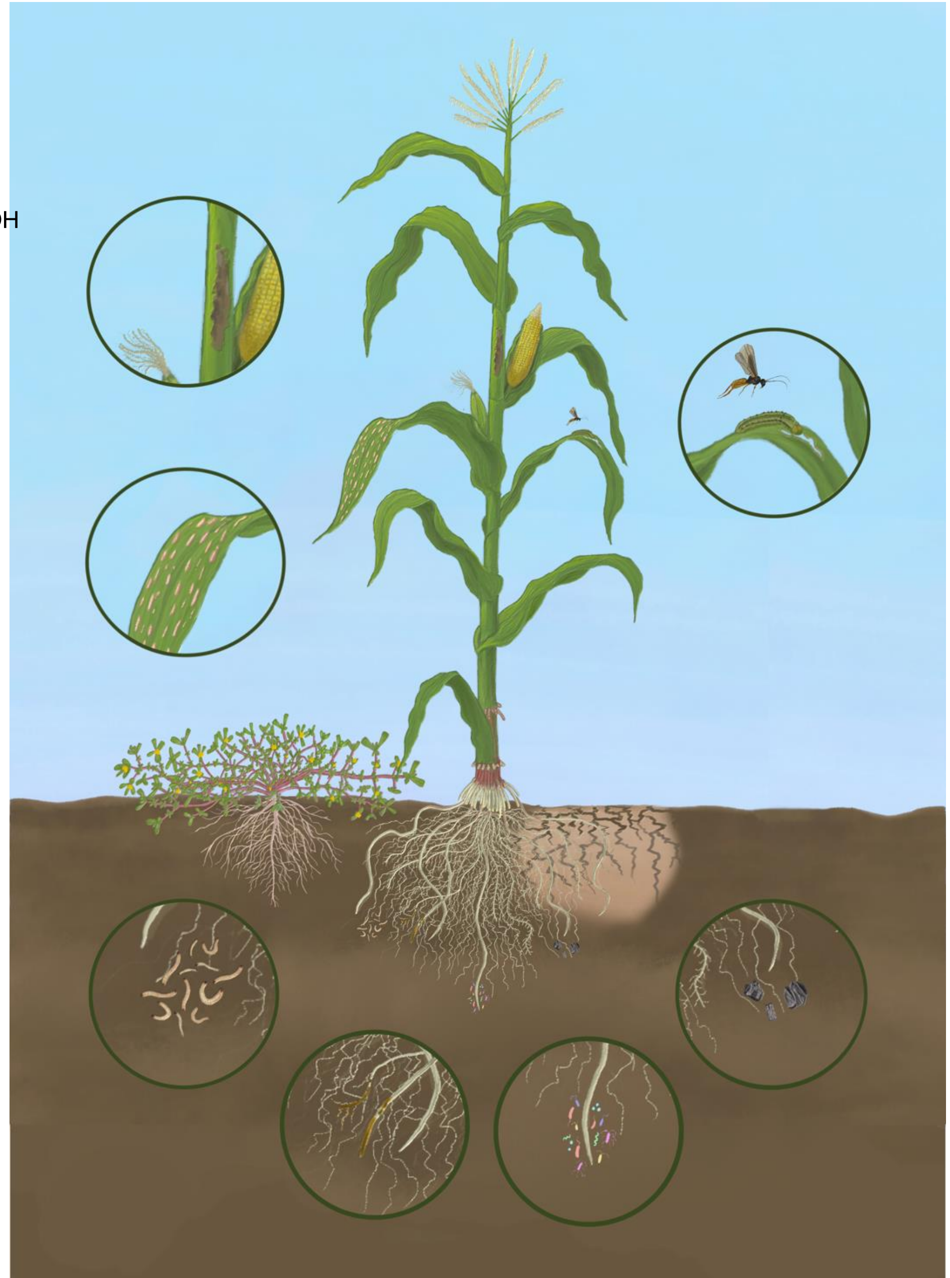




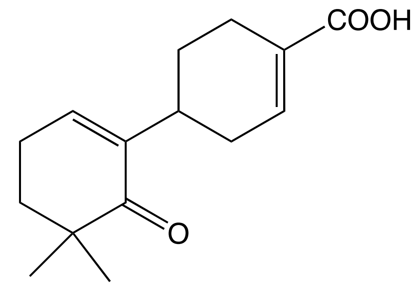
Oxylipins

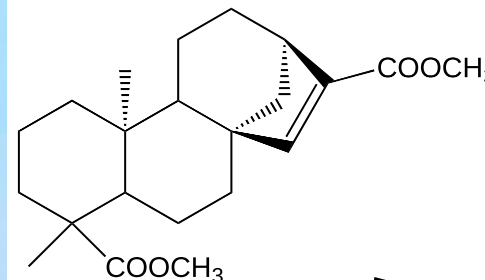


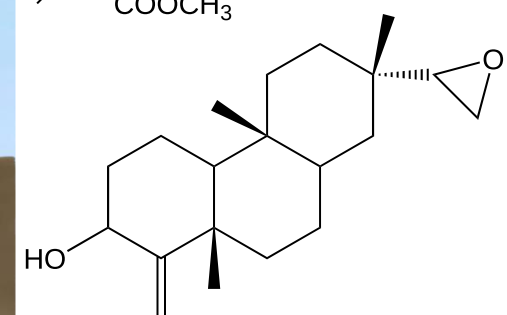
Benzoxazinoids

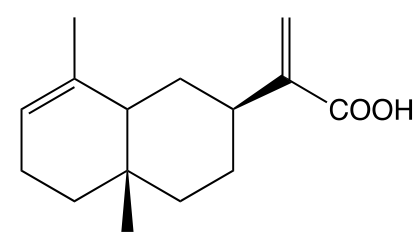


Terpenoids





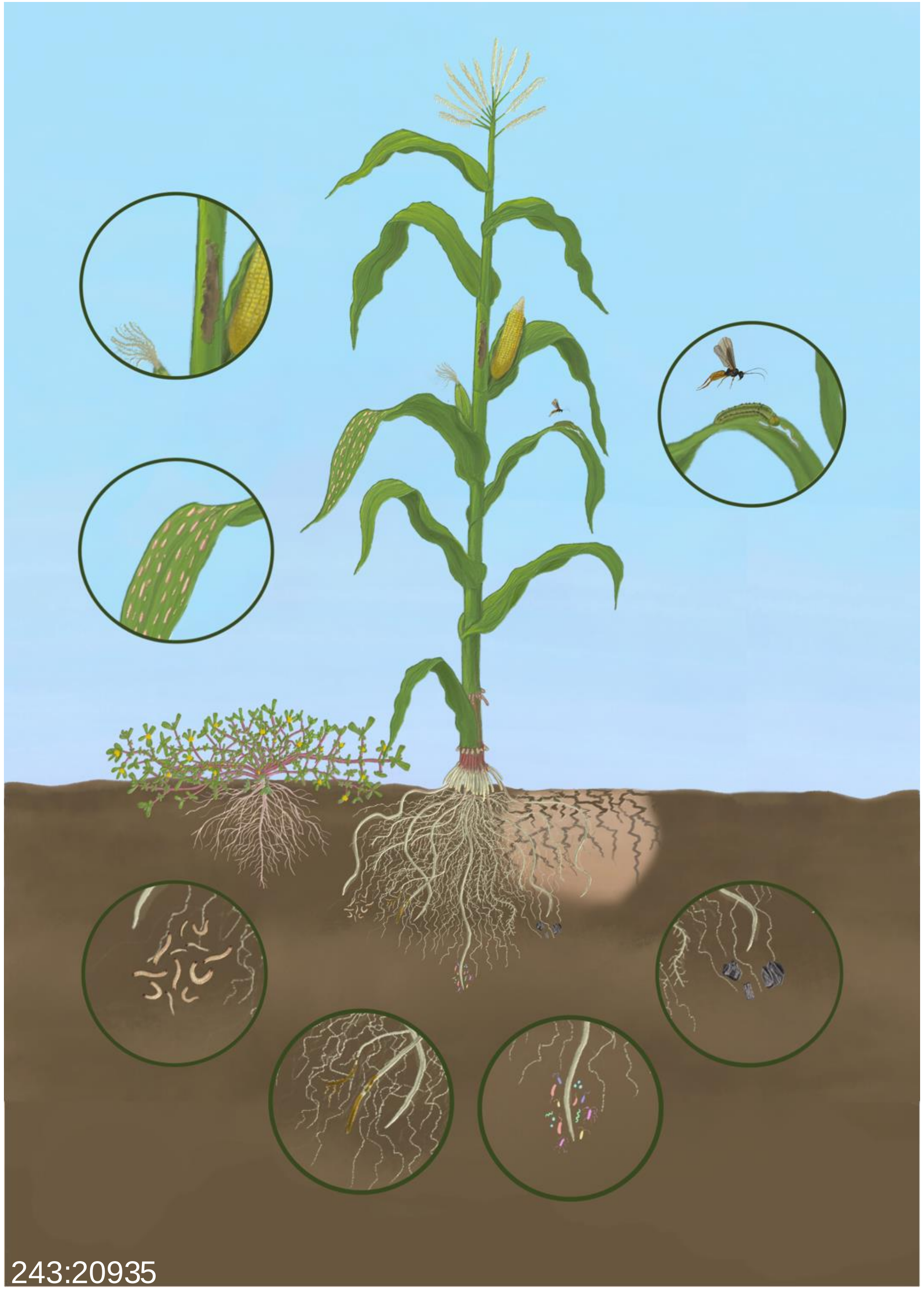




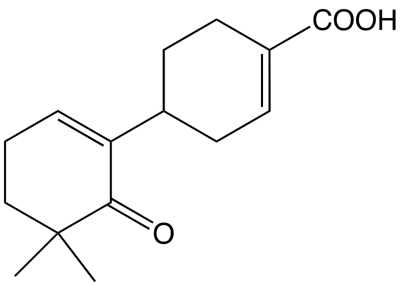
Yasmin et al. (2024) *New Phytol* 243:20935

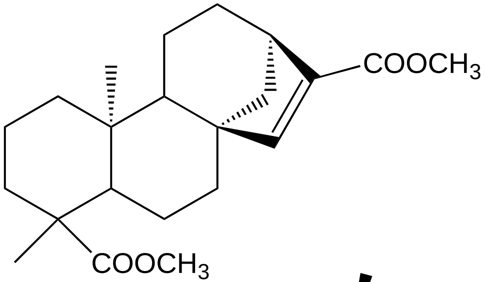
Maize produces a complex blend of chemical defense compounds

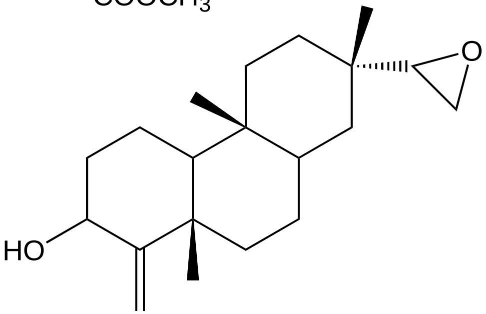
Collaboration with
Eric Schmelz (UC SD)

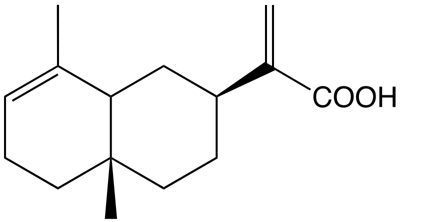


Terpenoids



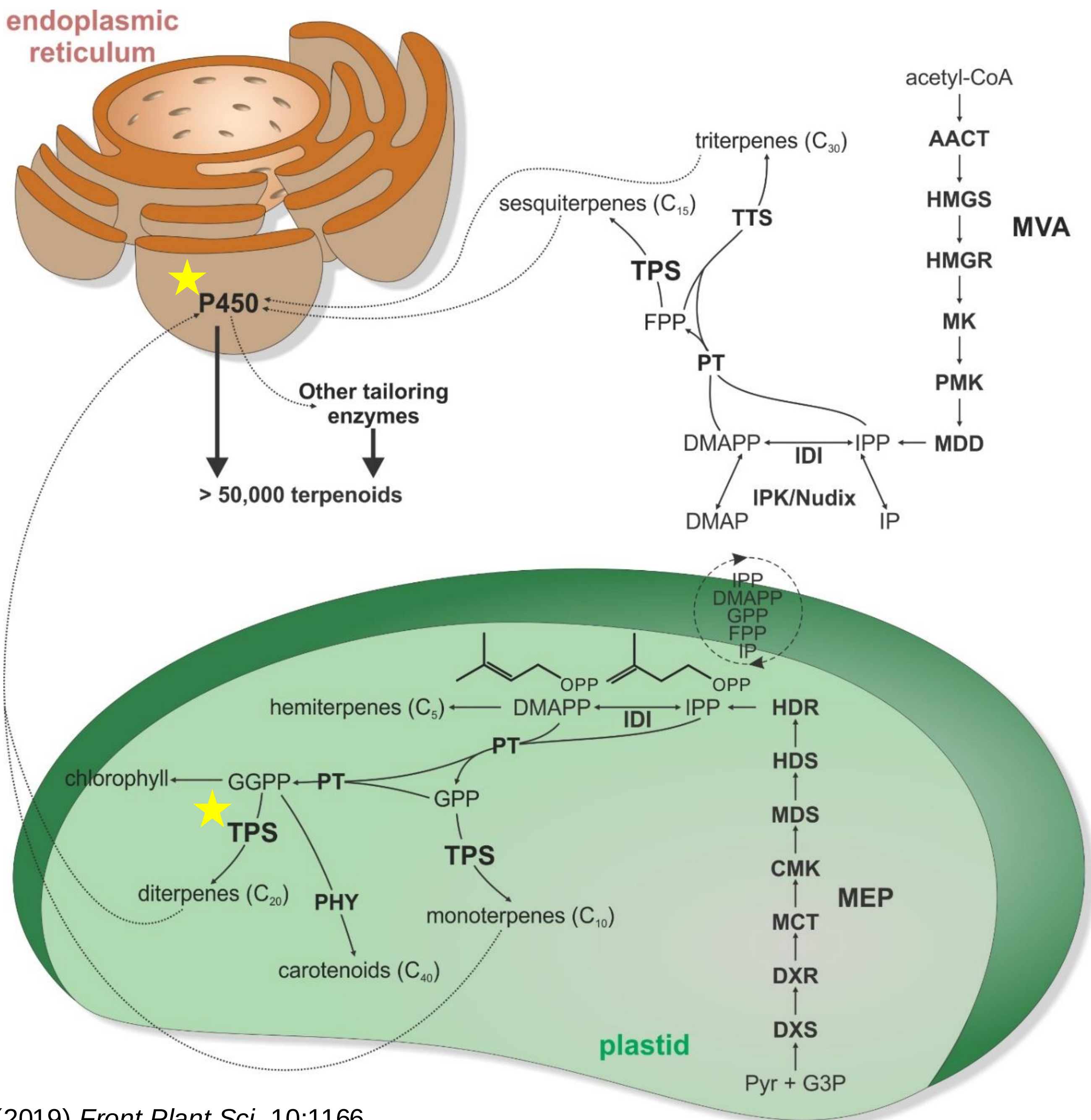






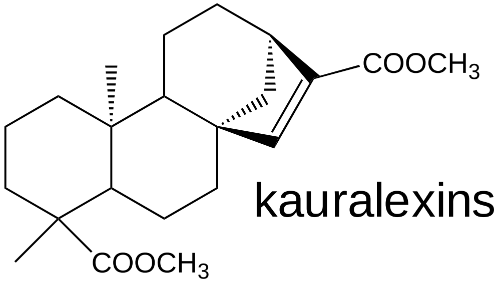
Yasmin et al. (2024) *New Phytol* 243:20935

Terpene synthases and cytochrome P450s are key drivers of terpenoid chemical diversity

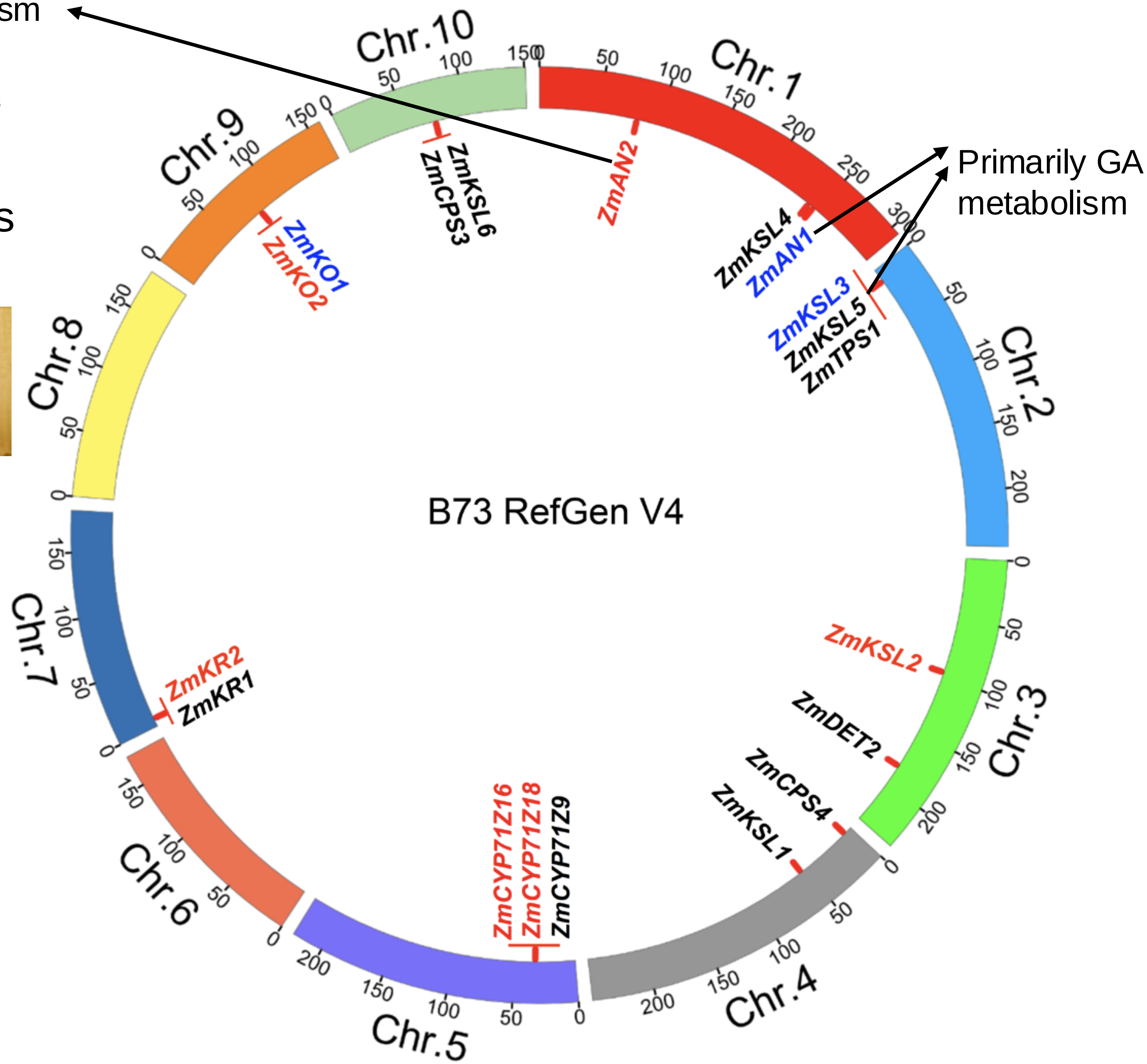


Karunanithi & Zerbe (2019) *Front Plant Sci.* 10:1166

Defining the diterpenoid-metabolic network in maize



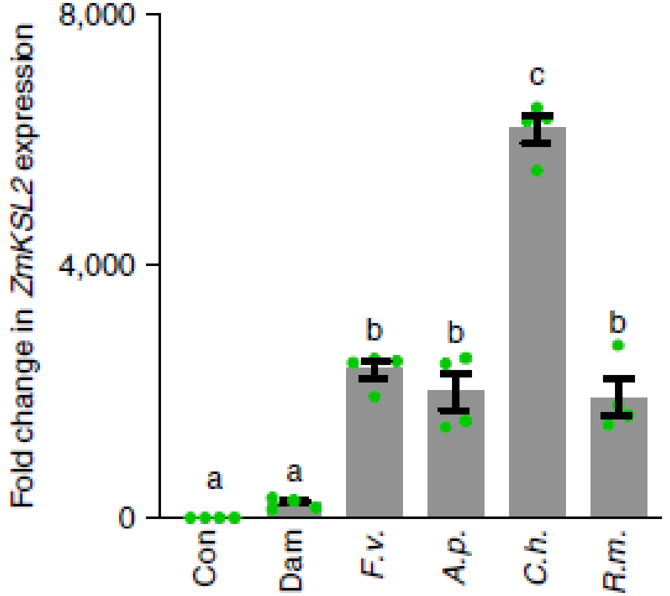
kauralexins



Ding et al. (2019) *Nat Plants* 5:1043-56

Genomics-enabled discovery of the kauralexin pathway

Transcriptomics show pathogen-elicited expression of diTPS gene candidates



Mutual rank analyses show patterns of diTPS transcript co-expression

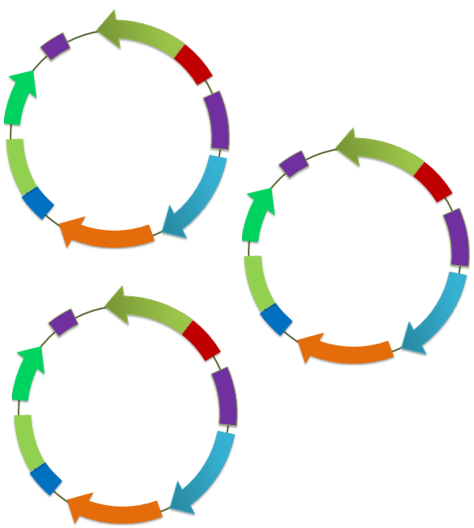
Specialized metabolism | GA metabolism

1 – 93	*ZmKSL2*	*ZmCYP71Z16*	*ZmCYP71Z18*	*ZmKO2*	*ZmKR2*	*ZmKSL4*	*ZmAN1*	*ZmTPS1*	*ZmKSL3*	*ZmKSL5*	*ZmKO1*
ZmAN2	2	65	1	4	20	17	21517	18146	2951	17940	16236
ZmKSL2		93	1	5	27	39	25114	25106	2129	18002	15792

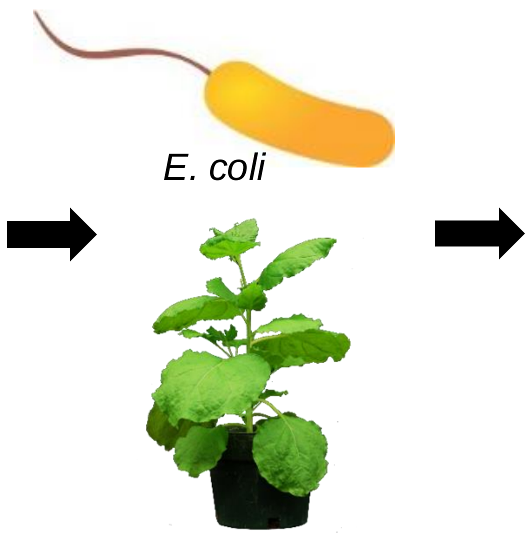
Ding et al. (2019) *Nat Plants* 5:1043-56

Combinatorial gene expression for efficient enzyme characterization

Multi-gene plasmids

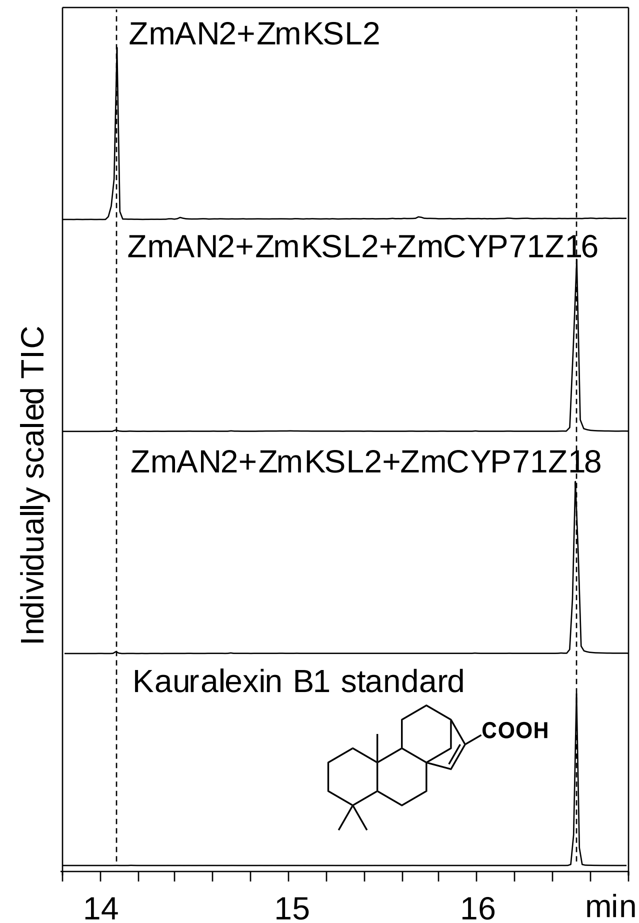


Co-expression



(*N. benthamiana*)

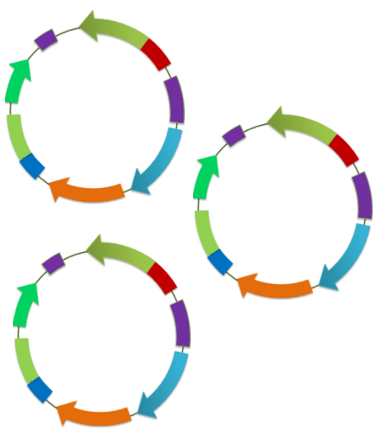
Metabolite profiling



Murphy et al. (2019) *J Vis Exp* 152. doi: 10.3791/59992

Functional characterization of the maize diterpenoid network

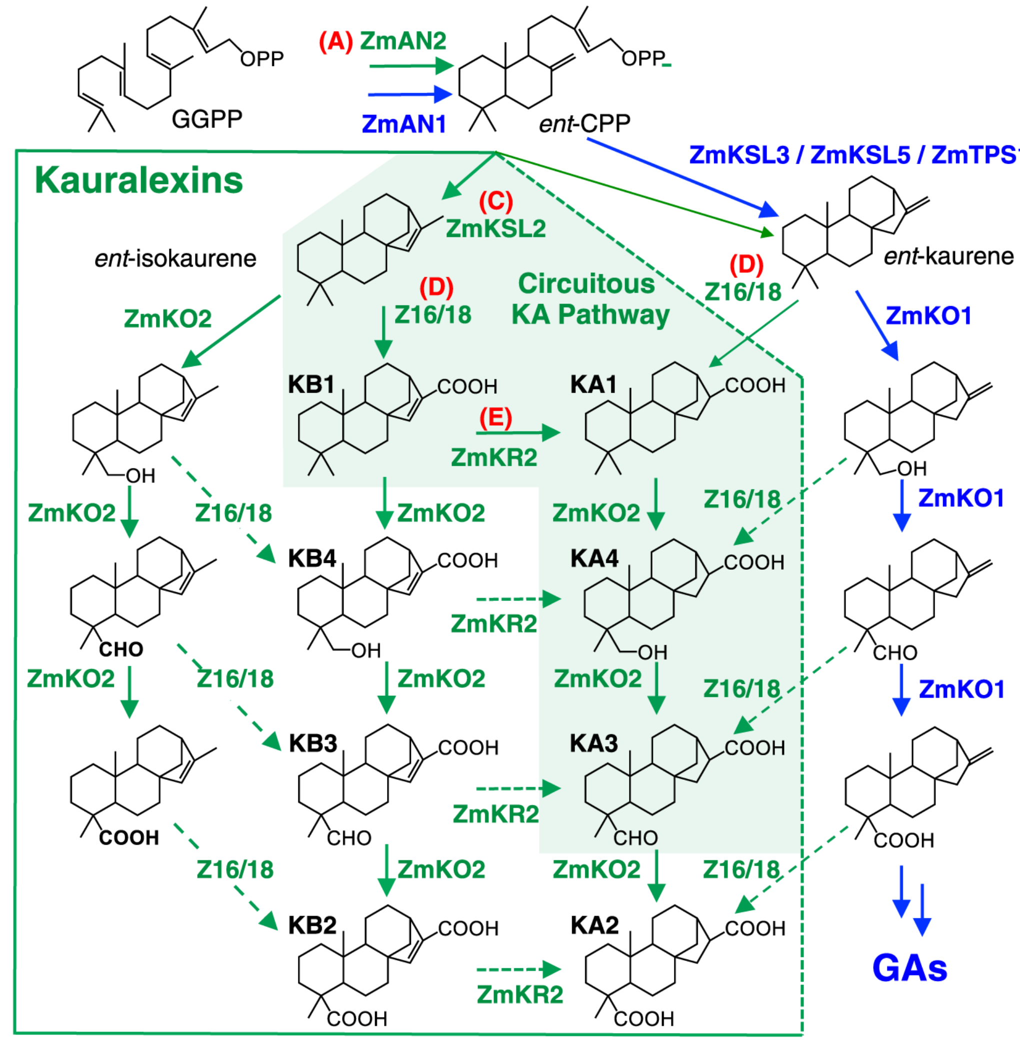
Multi-gene plasmids



Co-expression

E. coli

(*N. benthamiana*)

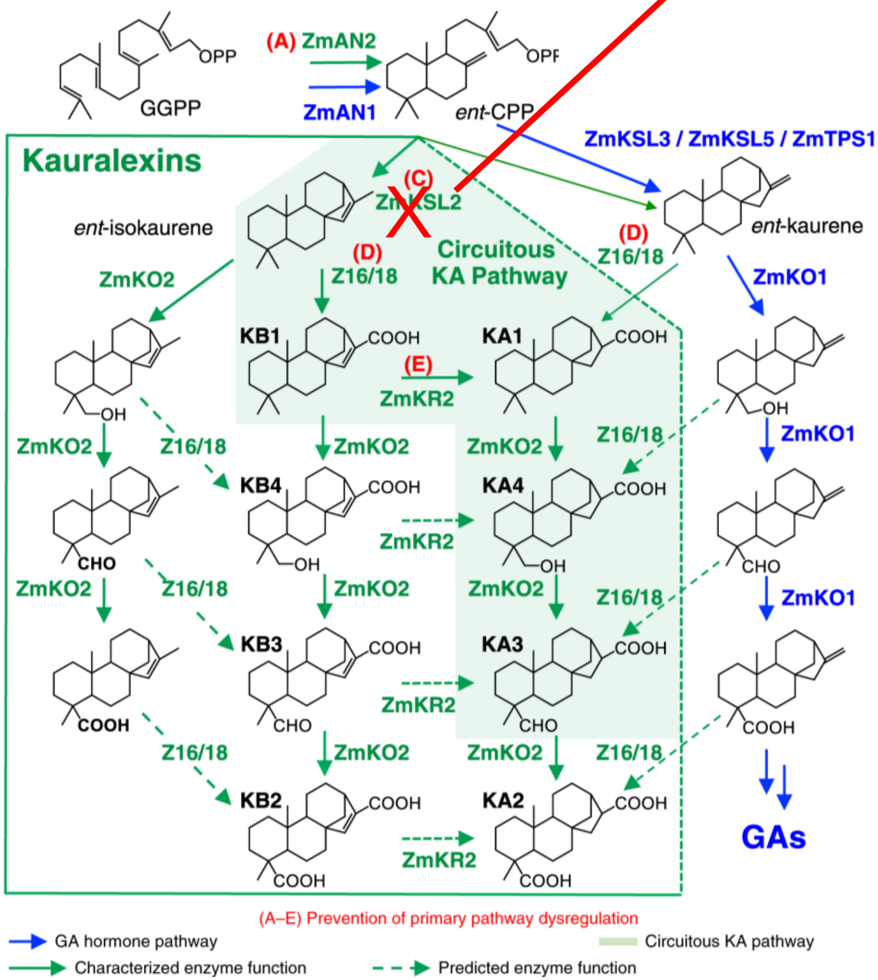


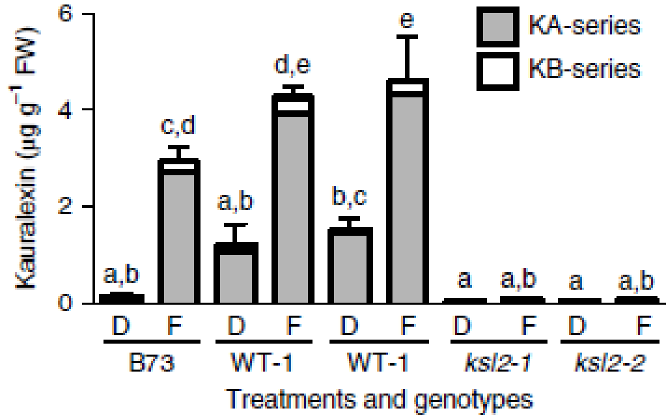
1 – 93	*Zm KSL2*	*ZmCYP 71Z16*	*ZmCYP 71Z18*	*Zm KO2*	*Zm KR2*	*Zm KSL4*	*Zm AN1*	*Zm TPS1*	*Zm KSL3*	*Zm KSL5*	*Zm KO1*
ZmAN2	2	65	1	4	20	17	21517	18146	2951	17940	16236
ZmKSL2		93	1	5	27	39	25114	25106	2129	18002	15792

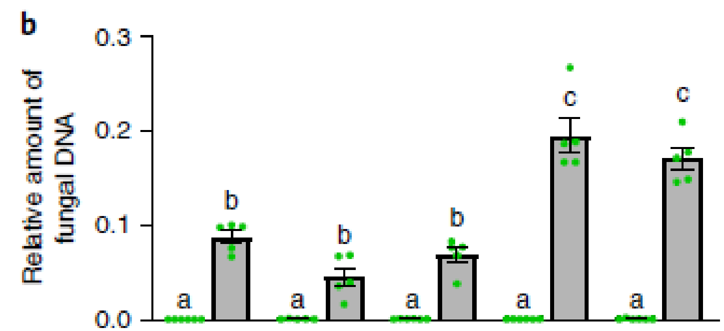
Ding et al. (2019) *Nat Plants* 5:1043-56

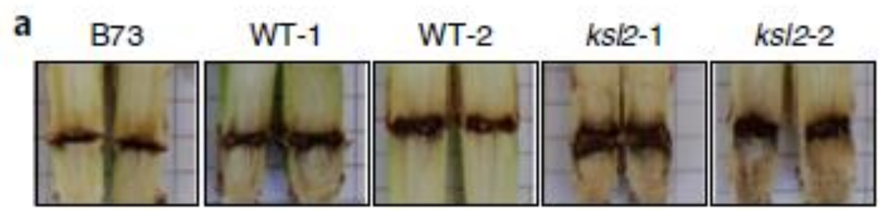
ZmKSL2 is critical for kauralexin biosynthesis and pathogen resistance

CRISPR/Cas9-enabled Zmksl2 mutant is deficient in kauralexins and more susceptible to fungal pathogens









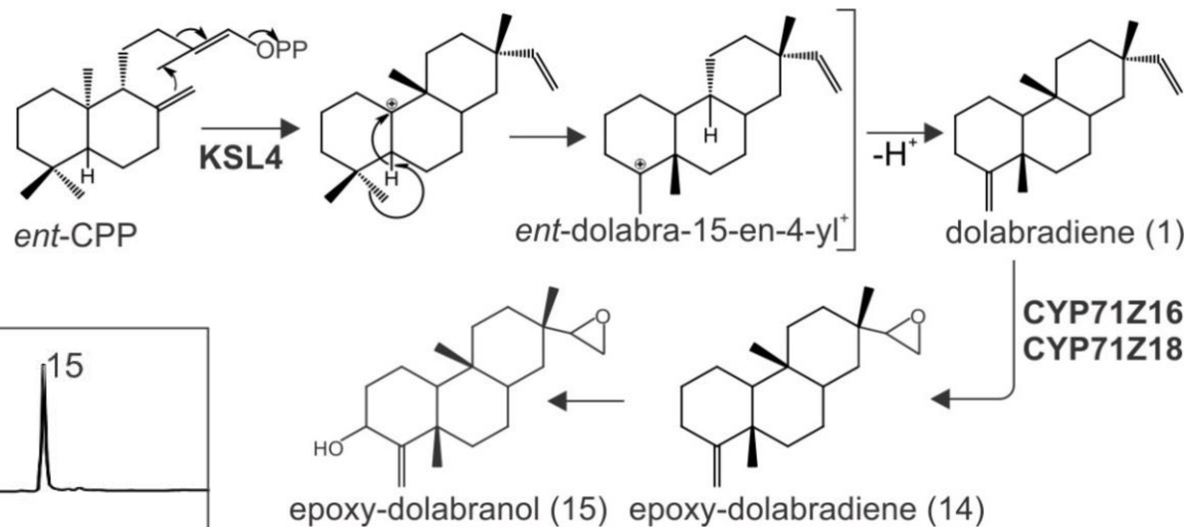
Ding et al. (2019) *Nat Plants* 5:1043-56

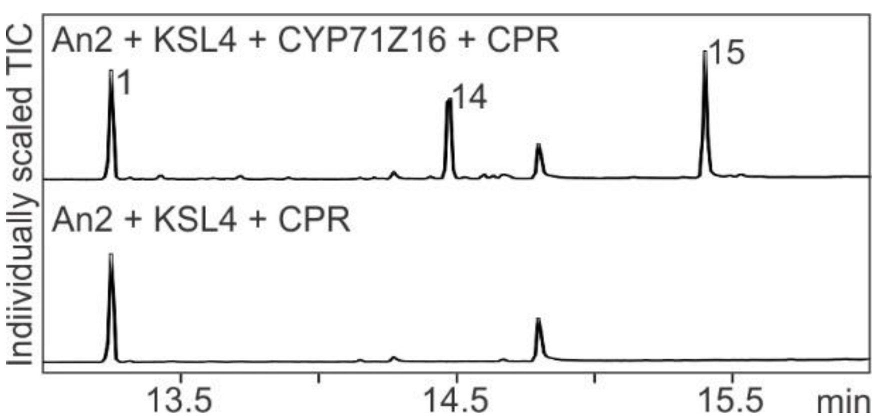
Discovery of the maize dolabralexin pathway

Mutual rank analyses show patterns of TPS and P450 co-expression

1–93	Zm KSL2	ZmCYP 71Z16	ZmCYP 71Z18	Zm KO2	Zm KR2	Zm KSL4	Zm AN1	Zm TPS1	Zm KSL3	Zm KSL5	Zm KO1
ZmAN2	2	65	1	4	20	17	21517	18146	2951	17940	16236
ZmKSL2		93	1	5	27	39	25114	25106	2129	18002	15792

Functional characterization of ZmAn2 with ZmKSL4 and ZmCYP71Z16/18

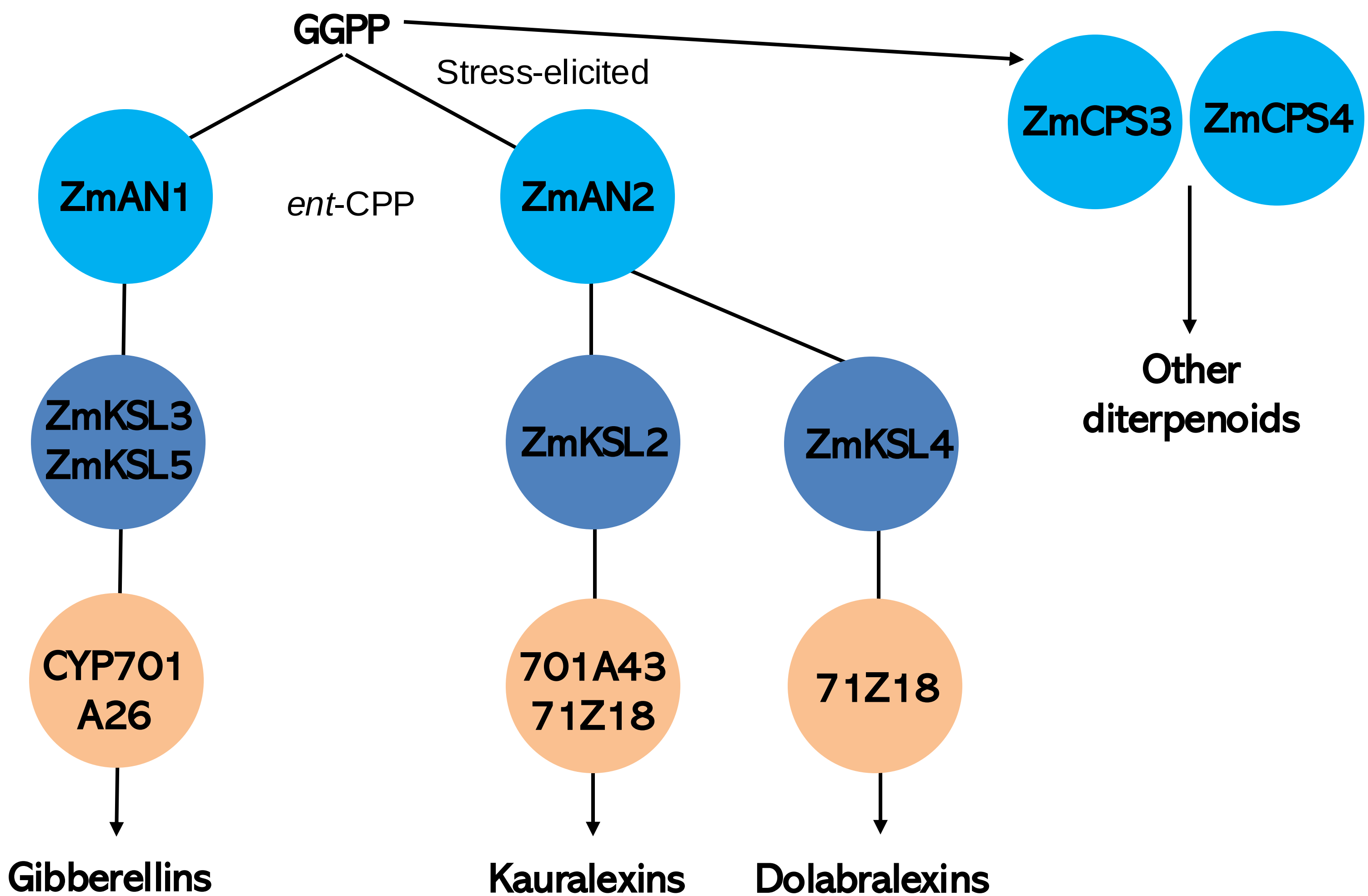




Mafu et al. (2018) *Plant Physiol* 176:2677-90. | Ding et al. (2019) *Nat Plants* 5:1043-56

Diterpenoid metabolism operates in form of modular pathway networks

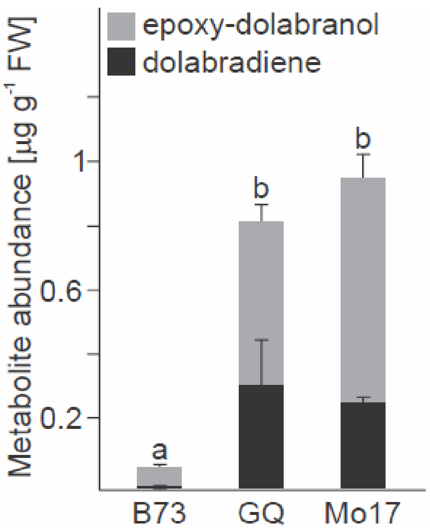
The functional divergence and differential expression of duplicated general metabolism genes minimizes dysregulation of hormone and defense pathway branches in maize.



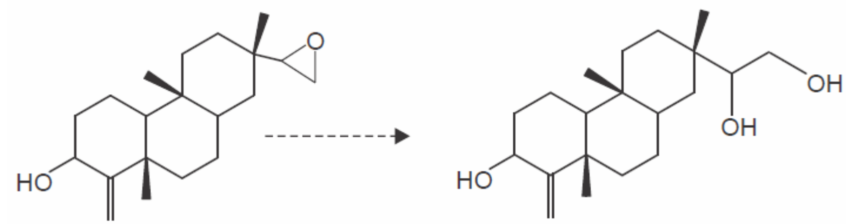
Yasmin et al. (2024) *New Phytol* 243:20935

Dolabralexins are abundant *in planta*

Identification of dolabralexins in root tissue of different field-grown maize genotypes

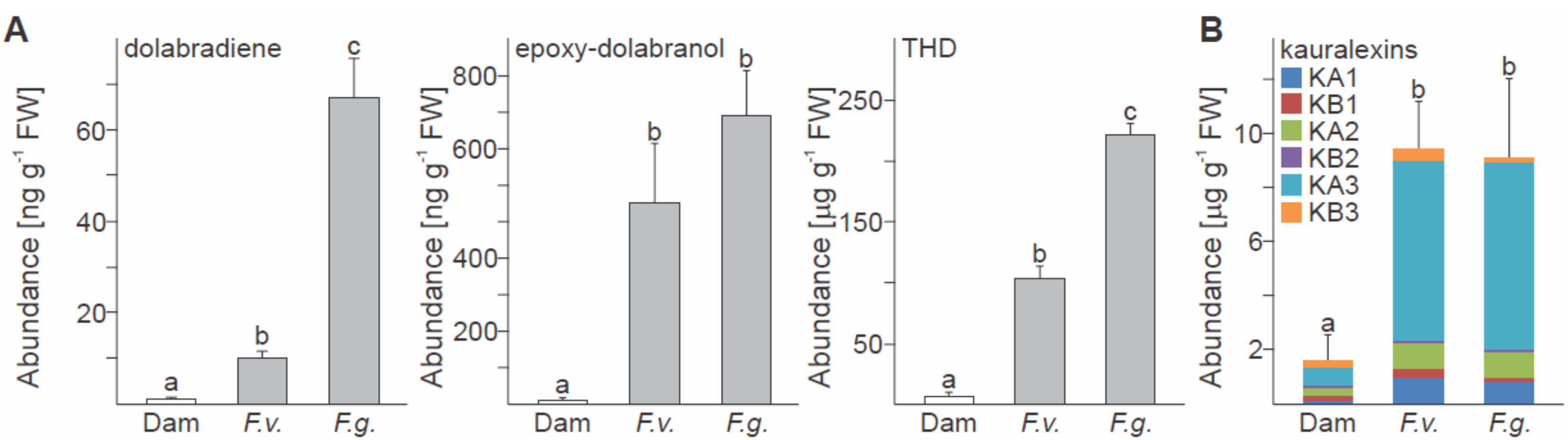


NMR analysis identifies additional dolabralexin metabolites in planta



epoxy-dolabranol **14** 3β,15,16-trihydroxydolabradiene **15**

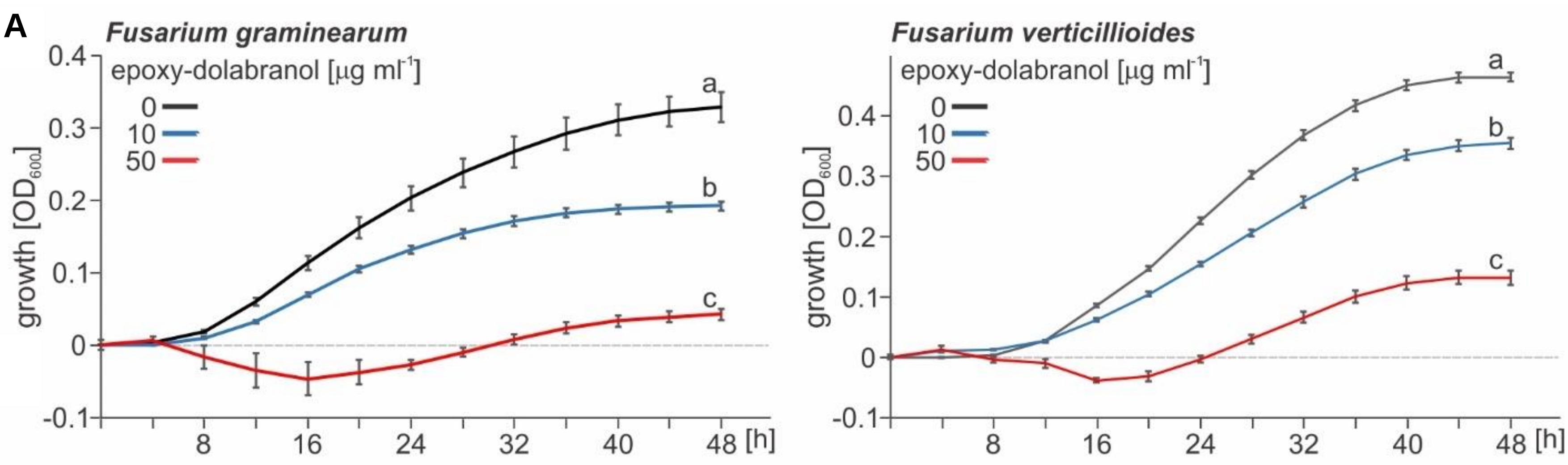
Metabolite accumulation in response to Fusarium pathogens



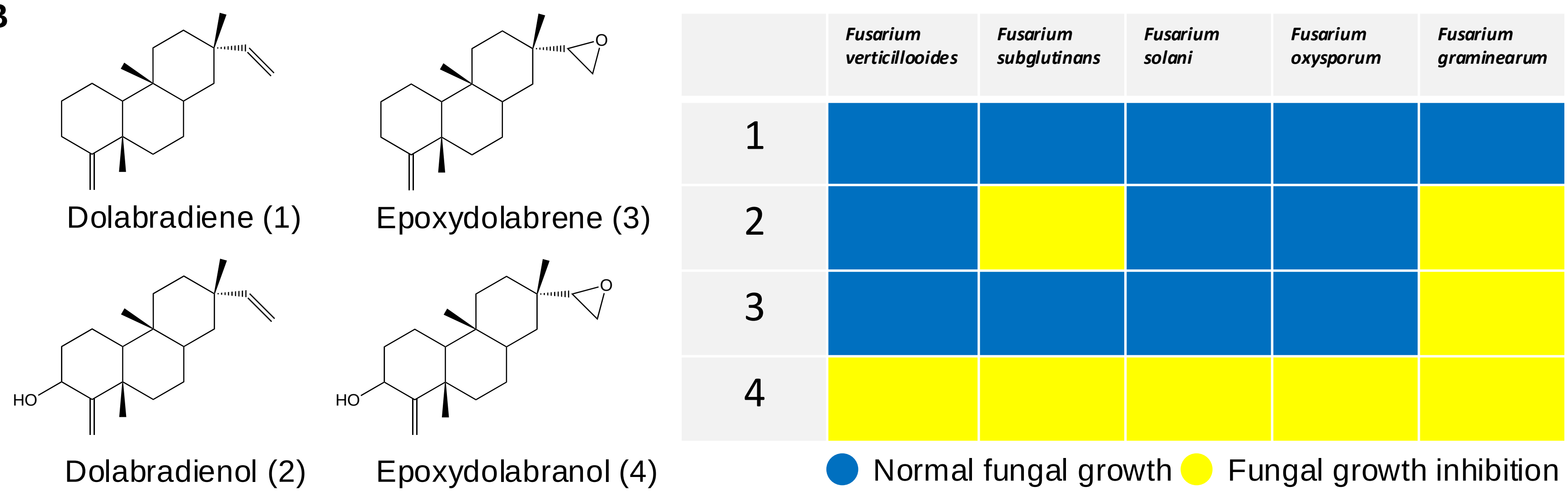
Mafu et al. (2018) *Plant Physiol* 176:2677-90.

Dolabralexins exhibit broad antifungal activity

A



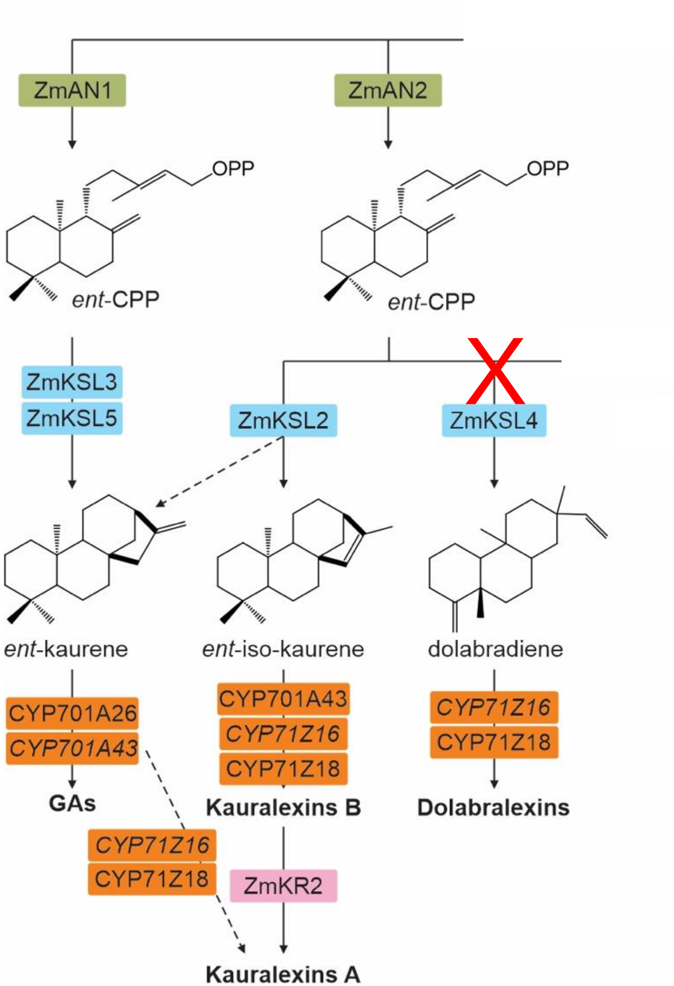
B

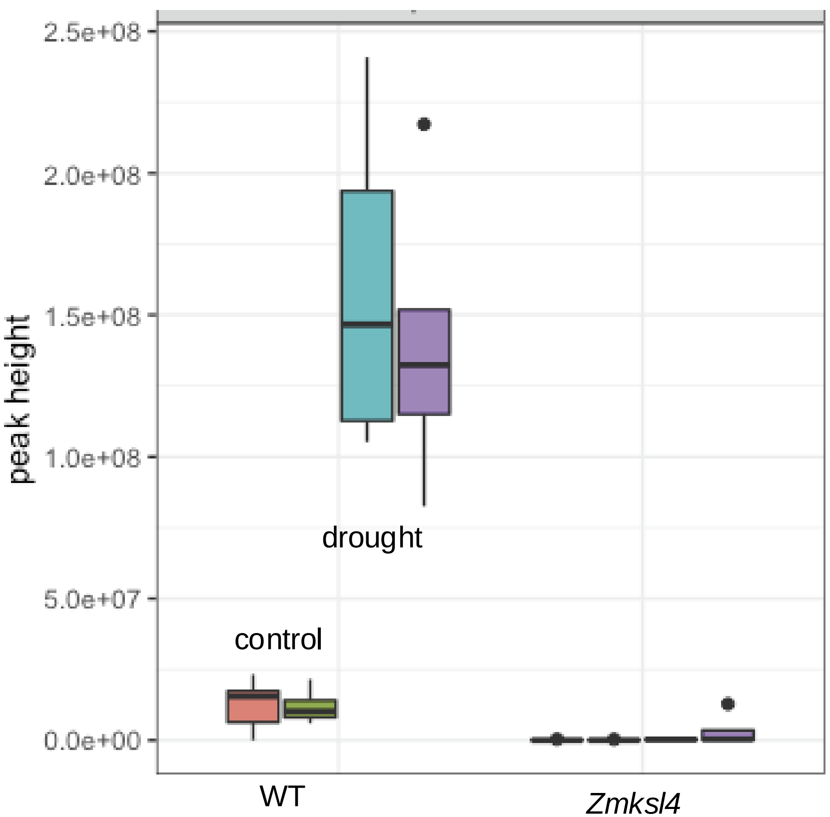


Murphy et al. unpublished

ZmKSL4 is the committed enzymes of dolabralexin biosynthesis

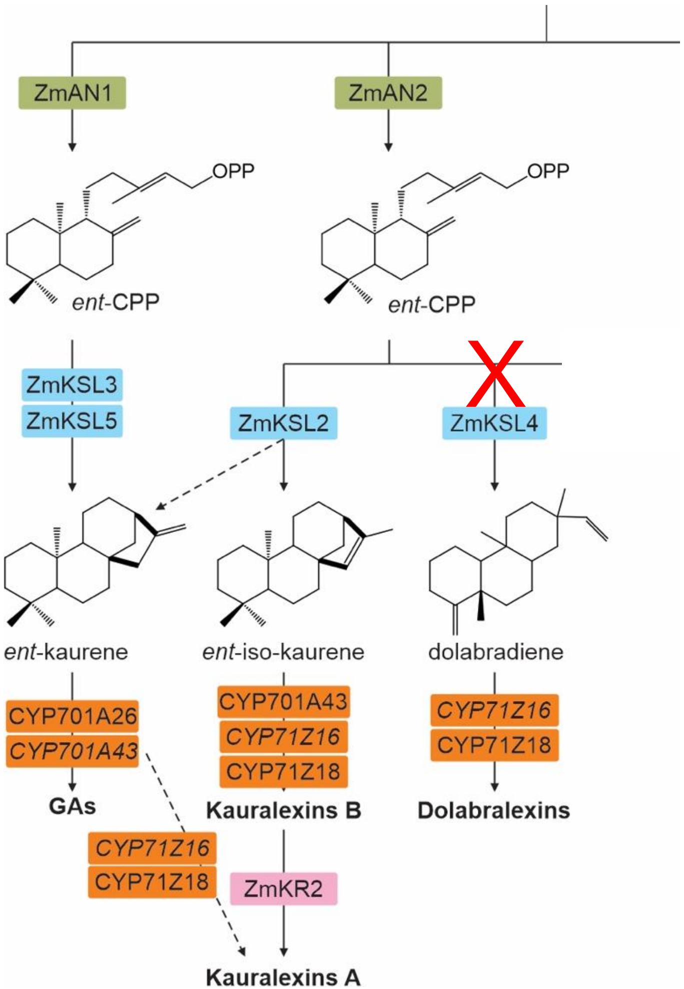
A CRISPR/Cas9 mutant identifies Zmksl4 as the committed step in dolabralexin biosynthesis

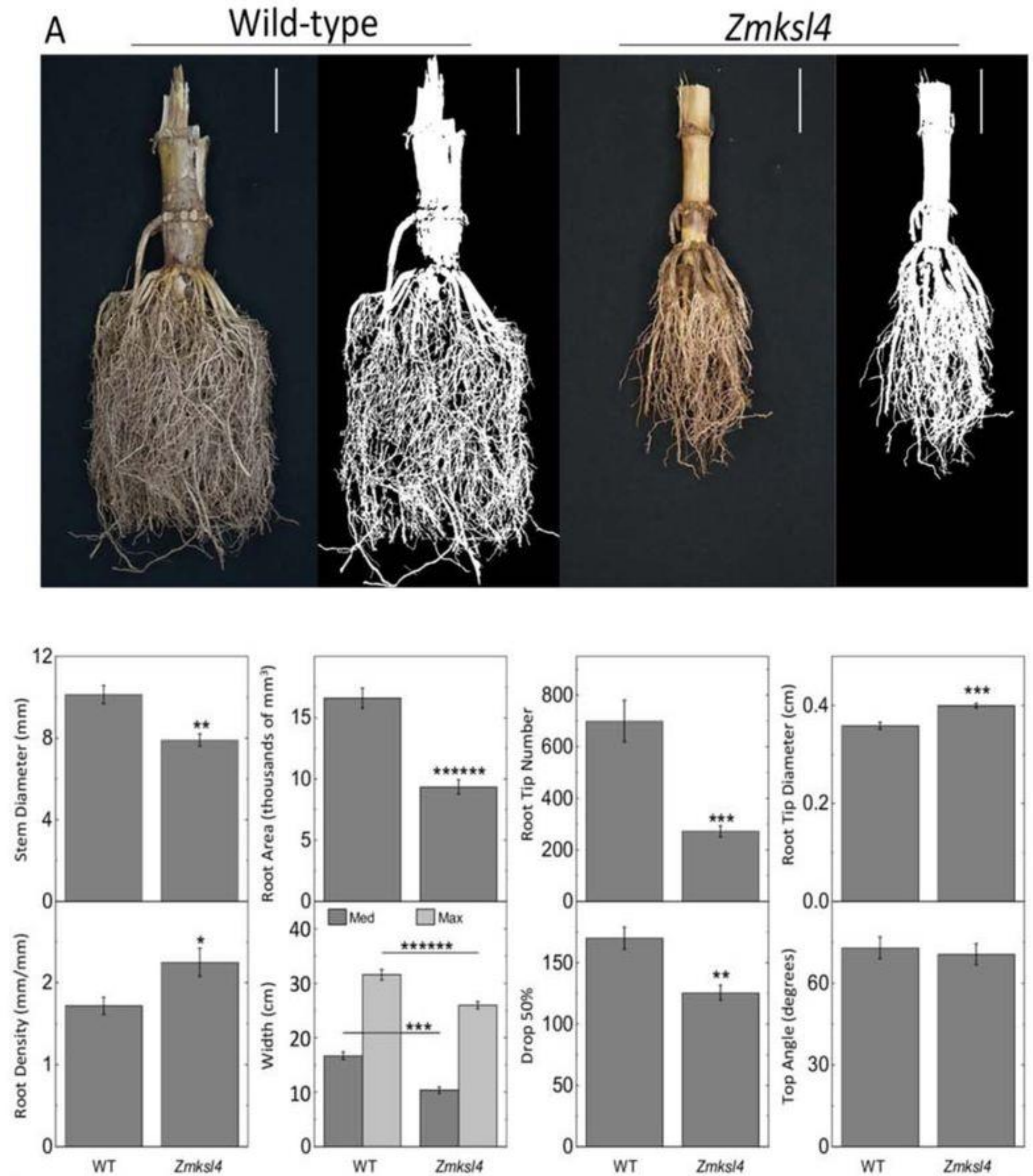




Murphy et al. (2023) *Plant Physiol* 192:1338-58

Zmksl4 mutants show altered root phenotypes under field conditions

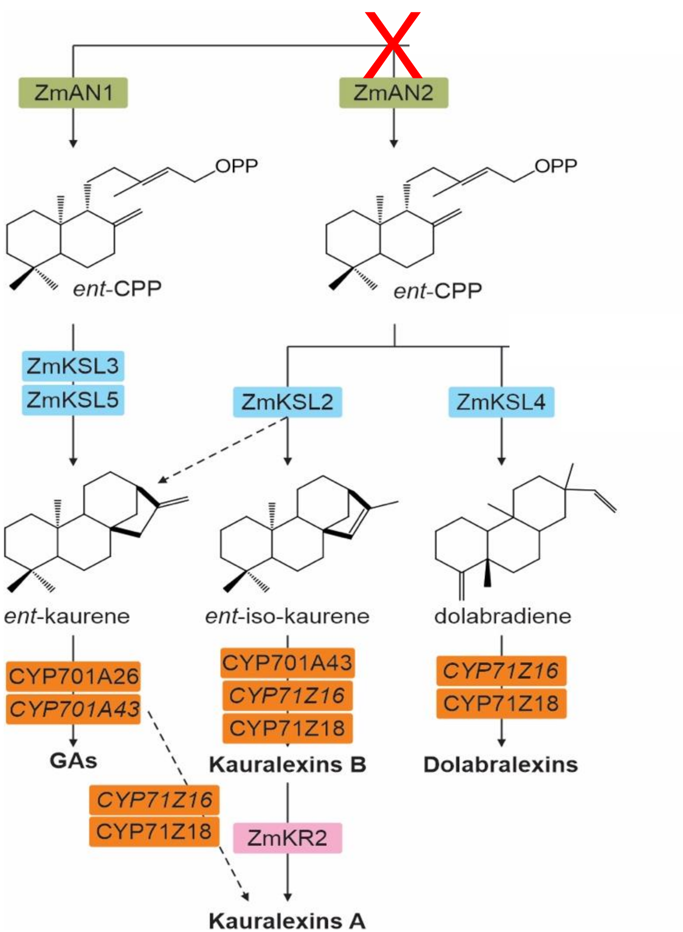


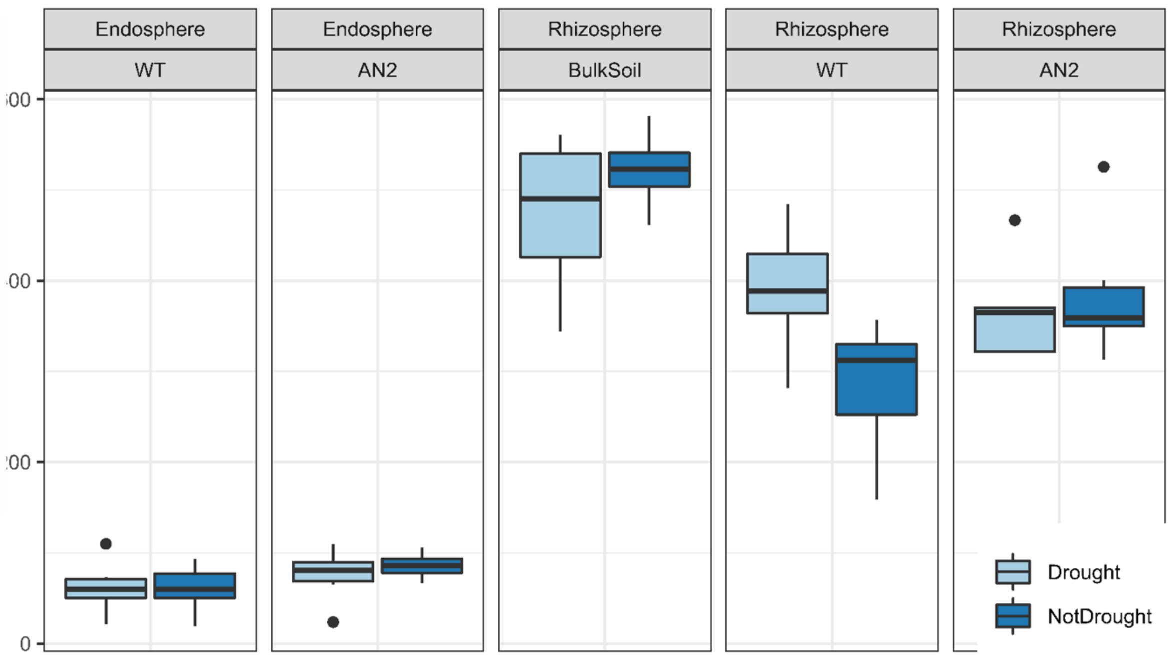


Murphy et al. (2023) *Plant Physiol* 192:1338-58

Dolabralexins have a possible role in root microbiome interactions

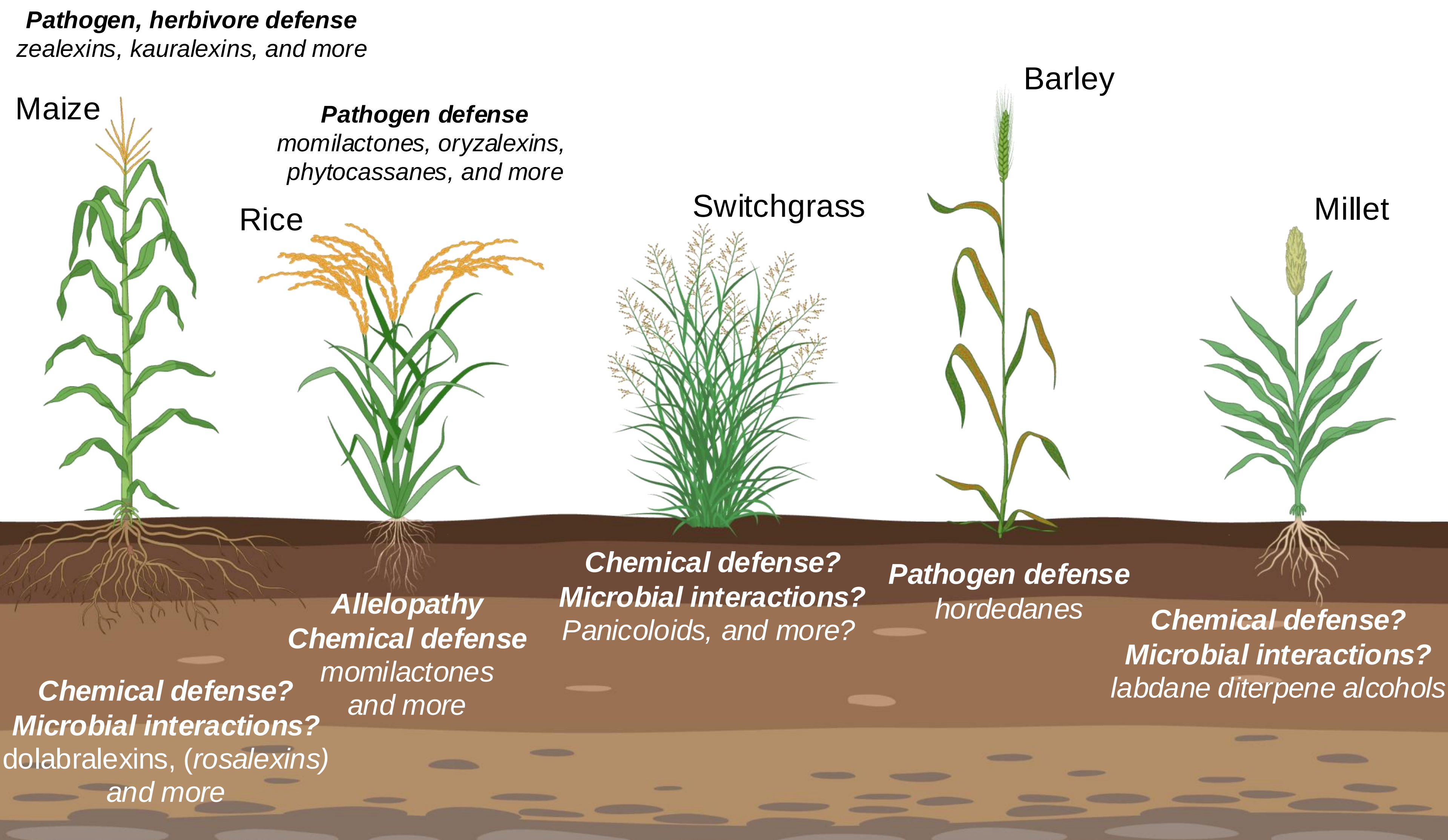
The root microbiome of wild type and diterpenoid-deficient Zman2 plants showed a distinct microbiome community composition under well-watered, but not drought-stress, conditions.





Murphy et al. (2021) *Sci Rep*, 11, 333

Species-specific blends of (di)terpenoids serve multiple roles in plant-environment interactions far beyond phytoalexin bioactivities.



Zerbe Lab Focus

Knowledge of the biosynthesis and function of plant terpenoid metabolites in plant growth and environmental adaptation can aid the development of new strategies for crop improvement and bioproduct engineering

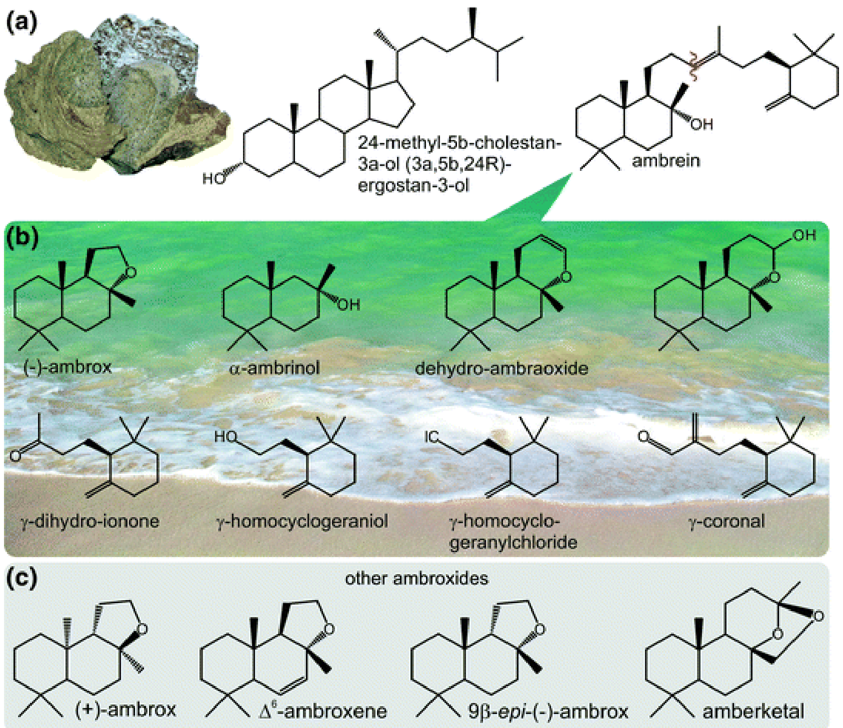
Terpenoid-mediated stress resilience in food and bioenergy crops

Defining terpenoid aroma metabolism for crop breeding

Enzyme discovery and engineering toward terpenoid bioproducts

UCDAVIS
UNIVERSITY OF CALIFORNIA

Developing resources for terpene fragrances



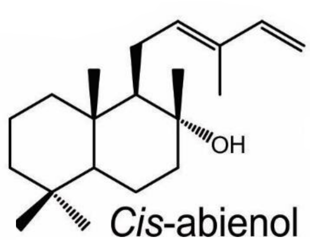
Ambergris, a natural secretion product of sperm whales was traditionally used in perfume manufacture

Sclareol and *cis*-abienol are plant-derived ambrox precursors

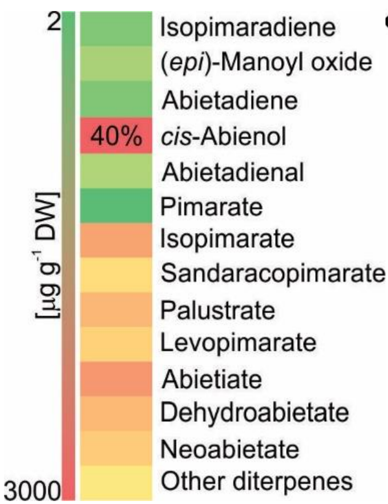

Balsam fir
Abies balsamea

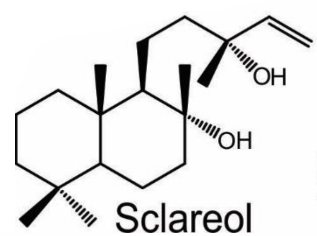
Clary sage
Salvia sclarea

cis-abienol is the major metabolite in bark tissue



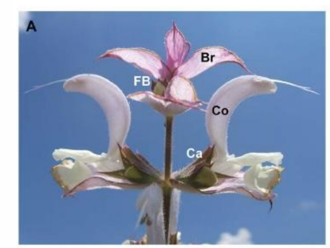
Cis-abienol

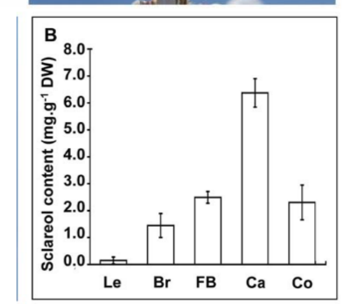




Sclareol

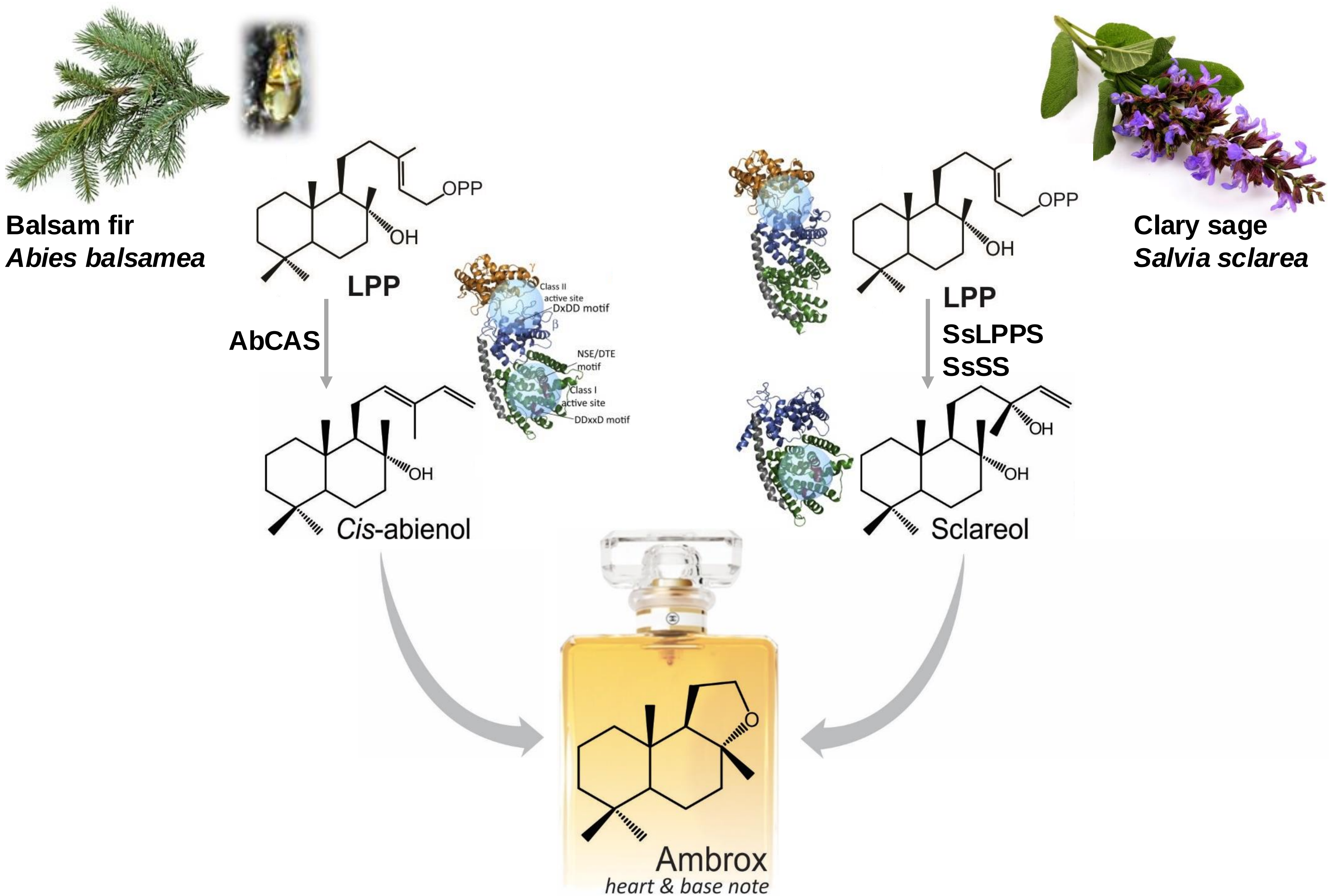
sclareol is the major metabolite in calyx tissue





Caniard *et al*., (2012) *BMC Plant Biol.* 12, 119
Zerbe *et al*. (2012) *J. Biol. Chem*. 277, 12121
Caissard *et al*., 2012 *PLoS ONE 7:e48253*

Sclareol and *cis*-abienol biosynthesis

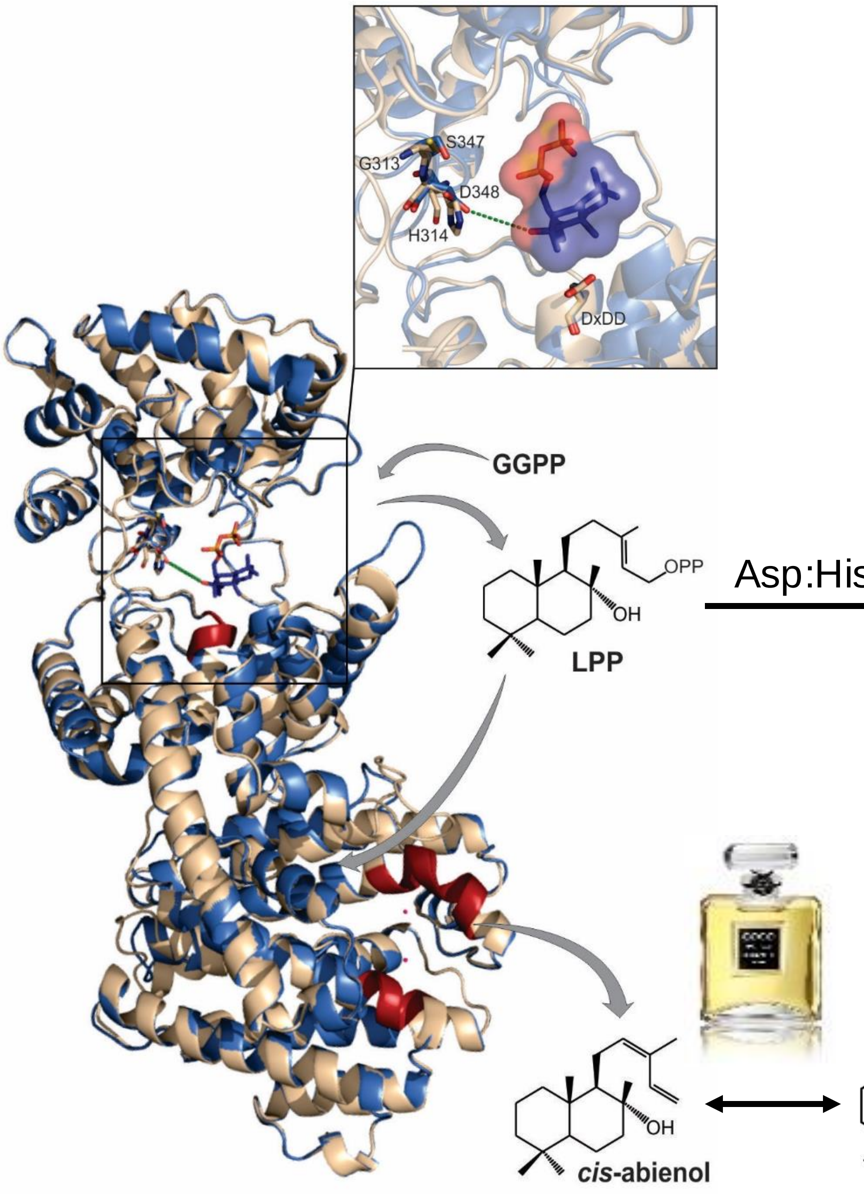


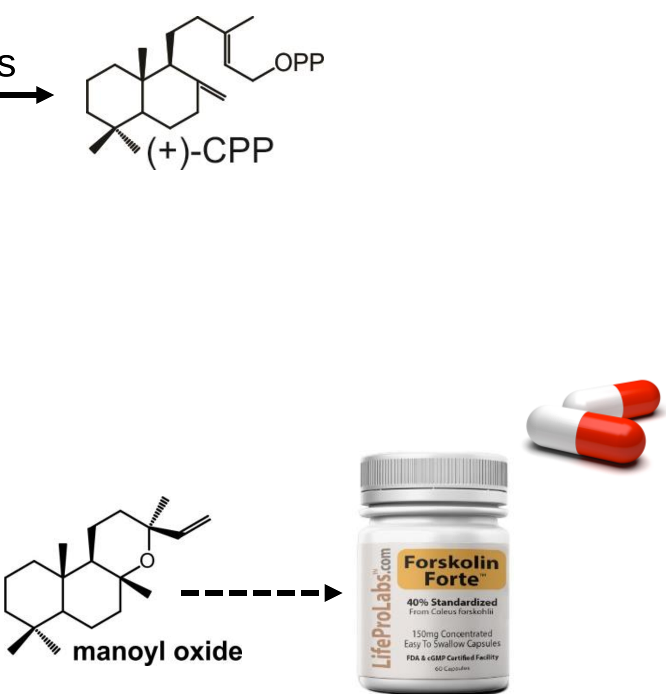
Caniard *et al*., (2012) *BMC Plant Biol.* 12, 119
Zerbe *et al*. (2012) *J. Biol. Chem*. 277, 12121

Cis-abienol synthase engineering toward new product outcomes

Balsam fir cis-abienol synthase

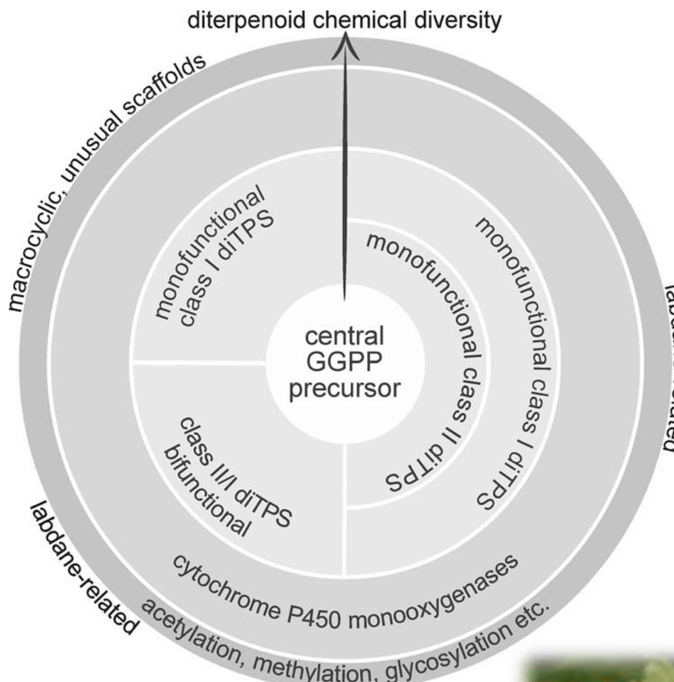
Terpene synthases show high catalytic plasticity enabling their vast evolutionary divergence - and protein engineering

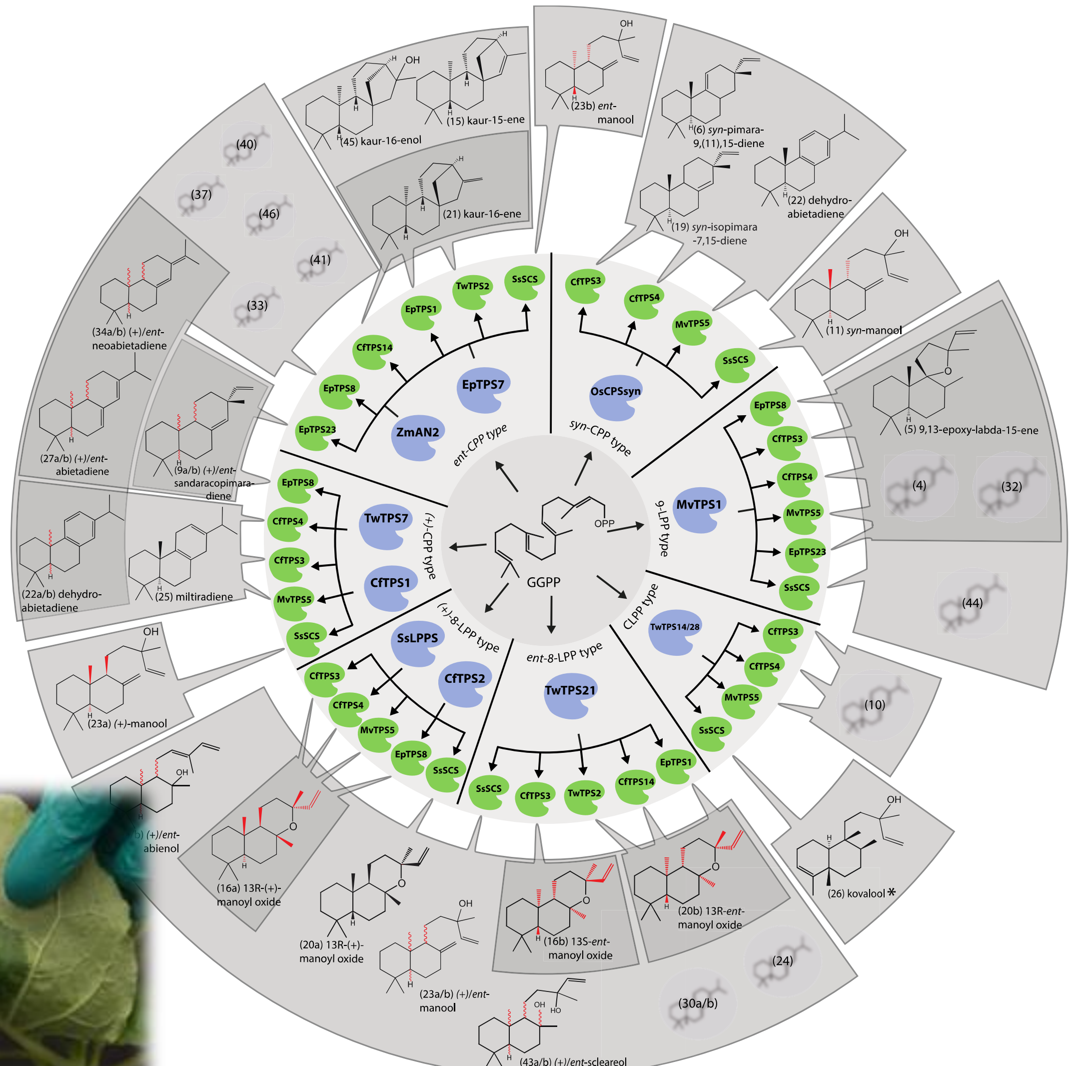




Harnessing modular diterpenoid pathways for engineering natural products

The modular pathway organization of plant diterpenoid metabolism enables the use of functionally distinct enzyme libraries for the combinatorial engineering of natural and new-to-nature bioproducts.



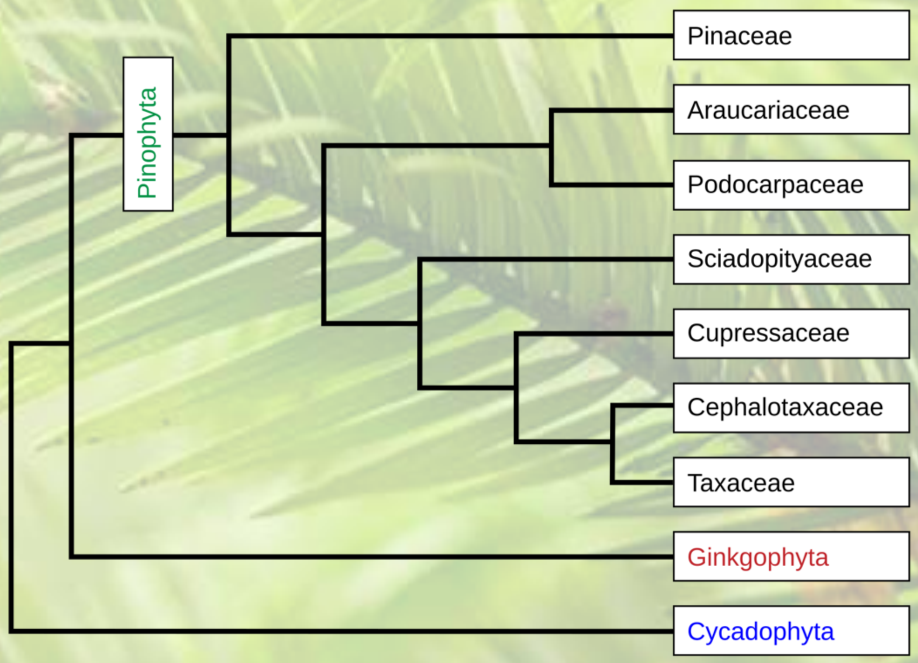


Andersen-Ranberg *et al.* (2016) *Angew. Chem.* 55: 2142-6

Collaboration with Björn Hamberger (MSU)

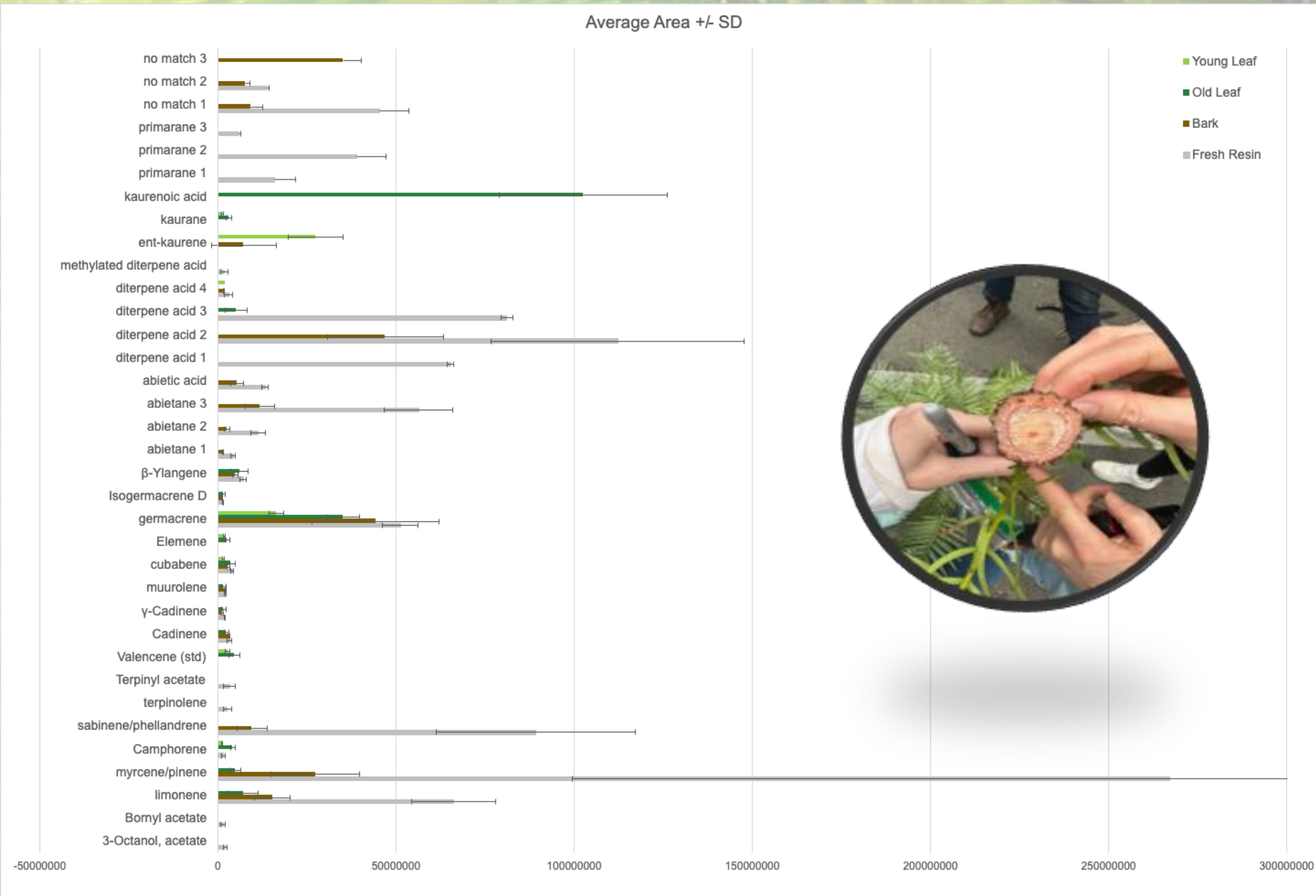
Exploring terpene metabolism in rare species – the 200M year old Wollemi pine (*Wollemia nobilis*)

Collaboration with Claudia Vickers (QUT, Australia), Senseory Plants, and the UC Berkeley Botanical Garden.



Exploring terpene metabolism in rare species – the 200M year old Wollemi pine (*Wollemia nobilis*)

Collaboration with Claudia Vickers (QUT, Australia), Senseory Plants, and the UC Berkeley Botanical Garden.



Experiential learning through plant biotechnology

Course-based Undergraduate Research Experience (CURE) course: Students learn modern biotechnology to explore and engineer plant metabolism.

Top: Elissa Nakano, Ethan Wendell, Anna Cowie, Jedidiah Peek, David Hurd, Jenevieve Weissman, Dylan Parker, Ayna Muftic
Bottom: Gabby Wyatt, Farida Yasmin, Mary Madera, Alex Gueorguieva, Samantha Herzig

New members: Siena Schumaker, Diana Paola Ochoa Vasquez, Iris Ramirez, Peyton Hilfird, Shamita Bhattacharjee, Shay Cihlar, Vannah Algador, Danielle Levi

Alumni with major contributions to this work: Lucas Crispi Cunha, Janessa Destremps, Kira Tiedge, Sibongile Mafu, Andrew Muchlinski, Meirong Jia, Katie Murphy, Hana Minsky

Collaborators: Eric Schmelz (USD), Alisa Huffaker (UCSD), Chris Topp (Danforth), Trent Northen (UCB), Dean Tantillo (UCD), Jörg Bohlmann (UBC), Robert Last (MSU), Björn Hamberger (MSU), Dan Major (Bar-Ilan University), Steve Knapp (UCD), Claudia Vickers (University of Queensland)

Funding Support

Department of Energy · NSF · National Institutes of Health · JGI Joint Genome Institute, Department of Energy · UC Davis Innovation Institute for Food and Health · USDA · California Strawberry Commission · Hellman Fellows Fund