

Syllabus: Fall 2016 – BCHM/HORT 640

Course Name: Plant Metabolic Biochemistry (Metabolic Plant Physiology)

Goals: This 3 credit, 15 week course will be taught by David Rhodes and Josh Widhalm. Students will be introduced to basic principles of plant metabolism including the mechanisms of uptake of small molecules from the environment and their elaboration into increasingly more complex structures via primary and secondary metabolism. The basic principles of enzymology, metabolite analysis and isotopic labeling will be discussed as a prelude to considering specific metabolic pathways. A major emphasis will be placed on plant carbon metabolism (photosynthesis, respiration, lipid biosynthesis, carotenoid and terpenoid biosynthesis), nitrogen, sulfur and secondary metabolism in plants, and interconnections between these metabolic networks. The course will introduce students to the various tiers of regulation of these pathways and efforts to genetically engineer them to enhance plant productivity, increase plant stress tolerance, and improve nutritional quality and economic value of harvested organs.

Instructor: Josh Widhalm E-mail jwidhalm@purdue.edu

Instructor: David Rhodes E-mail drhodes@purdue.edu

When: Tuesdays and Thursdays 1:30 - 2:45 PM, Fall Semester

Where: HLA Room 222

Prerequisite(s): HORT 301 - Plant Physiology, or equivalent

Recommended Textbook:

"Biochemistry and Molecular Biology of Plants, Second Edition" (Buchanan BB, Gruissem W, Jones RL), American Society of Plant Biologists, Rockville, MD, 2015

Quizzes, Assignment and Grades:

The course will include 6 quizzes, each worth 25 points. During the course, students will give short team presentations on "Strategies for Genetic Manipulation of Photosynthesis", and "Genetic Engineering of Lipids", each worth 40 points. At the conclusion of the course, students will give an individual

presentation during a mini-symposium on a topic of their choice related to plant carbon, nitrogen, sulfur or secondary metabolism. This presentation will be worth 100 points. There will be 6 literature discussion periods. A maximum of $6 \times 20 = 120$ points will be awarded for contributions made during literature discussion periods. Maximum total points = 450.

Students scoring a total score of 375 - 450 points will be given a grade of A, further sub-divided as follows:

- 425 - 450 = A+
- 400 - 424 = A
- 375 - 399 = A-

Students scoring a total of 300 - 374 points will be given a grade of B, further sub-divided as follows:

- 350 - 374 = B+
- 325 - 349 = B
- 300 - 324 = B-

Students scoring a total of 225 - 299 points will be given a grade of C, further sub-divided as follows:

- 275 - 299 = C+
- 250 - 274 = C
- 225 - 249 = C-

Students scoring a total of 150 - 224 points will be given a grade of D, further sub-divided as follows:

- 200 - 224 = D+
- 175 - 199 = D
- 150 - 174 = D-

Students scoring a total of less than 150 points will be given a grade of F:

- Less than 150 = F

Date: August 23, 2016

Objectives:

- Introduction.
 - Provide an overview of course syllabus, resources, goals, assignments, and grades.
 - What is a plant? (Widhalm)
 - Evolution of flowering plants (angiosperms) (Rhodes)
-

HORT640: Lecture 2, Week 1

Lecture title: Principles of enzymology (Rhodes)

Date: August 25, 2016

Objectives:

- Introduction to metabolic networks
 - Enzyme catalysis, temperature and pH optima
 - Methods of analysis of reaction velocities
 - Concepts of K_m and V_{max}
 - Michaelis-Menten kinetics
-

HORT640: Lecture 3, Week 2

Lecture title: Principles of enzymology (contd.) (Rhodes)

Date: August 30, 2016

Objectives:

- Enzyme inhibition
 - Competitive versus non-competitive inhibition
 - K_i
 - Allosteric regulation
-

HORT640: Lecture 4, Week 2

Lecture title: Principles of metabolite analysis and metabolomics (Rhodes)

Date: September 1, 2016

Objectives:

- Separation and quantification of metabolites
 - Ion exchange chromatography
 - Thin layer chromatography
 - Gas chromatography and GC-MS
 - Liquid chromatography and LC-MS
-

HORT640: Lecture 5, Week 3

Lecture title: Probing metabolic pathways by in vivo isotopic labeling (Rhodes)

Date: September 6, 2016

Objectives:

- Examples using radioisotopes (^{14}C , ^{35}S)
 - Examples using stable isotopes (^{13}C , ^{15}N)
 - Simulation of isotopic labeling in steady-state
 - Simulation of isotopic labeling in non-steady-state; dynamic modeling
-

HORT640: Lecture 6, Week 3

Lecture title: Overview of photosynthesis (Widhalm)

Date: September 8, 2016

Objectives: To introduce some of the fundamental principles of photosynthesis, and to introduce some of the key players in the light and carbon reactions of photosynthesis.

HORT640: Lecture 7, Week 4

Lecture title: Photosynthesis: Light Reactions (Widhalm)

Date: September 13, 2016

Objectives: This Lecture will try to fill in some of the details of the light reactions of photosynthesis. Some topics to be considered in more detail will be:

- Antennae: auxiliary light-harvesting pigment protein complexes in the photosystems
 - Structures of the photosystems in relation to their function
 - Electron transport pathways in chloroplast membranes
 - The Q-cycle in the chloroplast
 - ATP synthesis
 - Oxidation of water
 - Photosynthetic inhibitors
-

HORT640: Lecture 8, Week 4

Lecture title: Carbon reactions in C3 plants (Widhalm)

Date: September 15, 2016

Objectives: This Lecture will focus on the carbon reactions of photosynthesis with special reference to C3 plants. Specific topics to be considered are:

- The Calvin Cycle - intermediates, enzymes, and stoichiometry
- Rubisco - structure, activity, regulation

- Light-linked regulation of other enzymes of photosynthesis
-

HORT640: Lecture 9, Week 5

Lecture title: Photorespiration (Rhodes)

Date: September 20, 2016

Objectives: This Lecture will focus on the reactions of photosynthesis in C3 plants when oxygen, rather than CO₂, is used as a substrate by Rubisco. Specific topics to be considered are:

- Rubisco - kinetics with respect to CO₂ and O₂
- Recycling of 2-phosphoglycolate via the photorespiratory carbon oxidation (PCO) cycle; involvement of 3 organelles
- The involvement of nitrogenous solutes in this recycling (photorespiratory N cycle)
- Genetic evidence for the operation of the PCO cycle in Arabidopsis
- Effect of temperature on photorespiration and quantum yield

Assignments: HORT640 Quiz 1

HORT640: Lecture 10, Week 5

Lecture title: Variations in mechanisms of CO₂ fixation (Rhodes)

Date: September 22, 2016

Objectives: This Lecture will focus on the variations in mechanisms of photosynthesis (CO₂ fixation) in the plant kingdom. Specific topics to be considered are:

- C4 plant photosynthesis
 - general mechanism
 - anatomy of a C4 leaf
 - energetic costs of concentrating CO₂
 - NADP⁺-malic enzyme C4 photosynthesis
 - NAD⁺-malic enzyme C4 photosynthesis
 - PEP carboxykinase C4 photosynthesis
 - light regulation of C4 pathway enzymes
 - Crassulacean Acid Metabolism (CAM); difference in regulation of PEP carboxylase between C4 and CAM plants
 - Algal and cyanobacterial CO₂-HCO₃⁻ pumps coupled with carbonic anhydrase
-

HORT640: Lecture 11, Week 6

Lecture title: Strategies for Genetic Manipulation of Photosynthesis team

presentations (Rhodes, Widhalm)

Date: September 27, 2016

Assignments: Strategies for Genetic Manipulation of Photosynthesis team presentations.

Working in teams of 3, choose a manuscript on strategies for genetic manipulation of photosynthesis (discuss this manuscript with Josh Widhalm or David Rhodes before developing your presentation). Each team will make a 15 minute PowerPoint presentation and will allow 2-5 minutes for questions and discussion. The presentation should include:

- a brief introduction which states the goals and hypothesis tested,
- a description of the metabolic modifications performed,
- a brief description of obtained results, an explanation in the case of an unexpected outcome, and possible future experiments to achieve the initial goal.

This assignment will be worth 40 points.

HORT640: Lecture 12, Week 6

Lecture title: Carbohydrate metabolism; sucrose and starch synthesis (Rhodes)

Date: September 29, 2016

Objectives: This Lecture will focus on carbohydrate metabolism with special reference to sucrose and starch synthesis and degradation. Focus will be on regulation of these processes in a C₃ mesophyll leaf (please note that regulation may differ in other cell types and organs ... these differences will be highlighted in this and subsequent Lectures). Specific topics to be considered are:

- Metabolite transporters between chloroplast and cytosol
 - Biosynthetic pathways leading to synthesis of starch and sucrose
 - Key enzymes of sucrose and starch synthesis and degradation, and their regulation
-

HORT640: Lecture 13, Week 7

Lecture title: Glycolysis (Widhalm)

Date: October 4, 2016

Objectives: This Lecture will focus on the reactions of glycolysis; reactions that convert hexose phosphates to the 3-carbon compounds PEP and pyruvate via triose phosphates (glyceraldehyde 3-P and dihydroxyacetone phosphate) in the

cytosol. Specific topics to be considered are:

- Intermediates and enzymes of glycolysis
- Regulation of PFK (ATP-dependent phosphofructokinase)
- Metabolic bypasses (adaptations to Pi-deficiency)
- Alcohol and lactate fermentation under anaerobic stress

Assignments: HORT640 Quiz 2.

HORT640: Lecture 14, Week 7

Lecture title: Citric acid cycle (Widhalm)

Date: October 6, 2016

Objectives

This Lecture will focus on the carbon reactions of the citric acid cycle (TCA cycle; Krebs cycle). Specific topics to be covered include:

- An overview of the general mechanism of oxidative phosphorylation in mitochondria.
 - Role of glycolysis and the citric acid cycle in producing intermediates for biosynthesis.
 - Conversions of PEP, pyruvate, oxaloacetate and malate.
 - The pyruvate dehydrogenase complex and its regulation.
-

October 11, 2016 No Class, October break

HORT640: Lecture 15, Week 8

Lecture title: Structure and function of lipids; fatty acid biosynthesis (Rhodes)

Date: October 13, 2016

Objectives: This Lecture will focus on the structure and function of plant lipids, with special emphasis on fatty acid biosynthesis. Specific topics to be covered include:

- Definition of the term "lipids" and their diverse roles in plants
 - Major classes of lipids; structures of fatty acids; nomenclature
 - Fatty acid biosynthesis; overview; compartmentation
 - Central role of acetyl-CoA and malonyl-CoA
 - Acetyl-CoA carboxylase (ACCase)
 - Fatty acid synthase (refers to all enzyme activities in fatty acid biosynthesis except ACCase)
-

HORT640: Lecture 16, Week 9

Lecture title: Fatty acid biosynthesis (contd.); synthesis of membrane lipids (Widhalm)

Date: October 18, 2016

Objectives: This Lecture will continue discussion of fatty acid biosynthesis, and then introduce the synthesis pathway for membrane lipids. Specific topics to be covered include:

- The terminal steps in fatty acid biosynthesis
- Desaturation and elongation of C16 and C18 fatty acids
- Unusual fatty acids; long-chain fatty acids
- The prokaryotic and eukaryotic pathways of glycerolipid synthesis; compartmentation
- Synthesis of membrane lipids; synthesis of phosphatidate; the CDP-diacylglycerol and diacylglycerol pathways of phospholipid synthesis

Assignments: HORT640 Quiz 3.

HORT640: Lecture 17, Week 9

Lecture title: Genetic engineering of lipids (Widhalm)

Date: October 20, 2016

Objectives: This Lecture will continue discussion of fatty acid biosynthesis, with specific emphasis on genetic/metabolic engineering of lipid metabolism. Specific examples of metabolic engineering include:

- Improvement of oil quality
 - Increasing oil yields
 - Industrial applications
 - High laurate
 - Ricinoleic acid
 - gamma-Linolenic acid
 - Biodegradable plastics
-

HORT640: Lecture 18, Week 10

Lecture title: Genetic Engineering of Lipids team presentations (Rhodes, Widhalm)

Date: October 25, 2016

Assignments: Genetic Engineering of Lipids team presentations.

Working in teams of 3, choose a manuscript on genetic/metabolic engineering of lipid metabolism (discuss this paper with Josh Widhalm or David Rhodes before developing your presentation). Each team will make a 15 minute PowerPoint presentation and will allow 2-5 minutes for questions and discussion. The presentation should include:

- a brief introduction which states the goals and hypothesis tested,
- a description of the metabolic modifications performed,
- a brief description of obtained results, an explanation in the case of an unexpected outcome, and possible future experiments to achieve the initial goal.

This assignment will be worth 40 points.

HORT640: Lecture 19, Week 10

Lecture title: Carotenoid biosynthesis (Widhalm)

Date: October 27, 2016

Objectives: This Lecture will focus on the synthesis of carotenoids; the pathway of synthesis is a branch of the isoprenoid pathway also involved in terpenoid biosynthesis to be considered in Lecture 20. Specific topics to be considered are:

- The mevalonate and non-mevalonate pathways of synthesis of isopentenylidiphosphate (IPP)
 - Synthesis of geranylidiphosphate from IPP
 - Production of phytoene (C40)
 - Synthesis of carotenoids from phytoene
 - Engineering of carotenoid biosynthesis (golden rice)
 - Abscisic acid synthesis and metabolism
-

HORT640: Lecture 20, Week 11

Lecture title: Terpenoids (Widhalm)

Date: November 1, 2016

Objectives: This Lecture will focus on the synthesis of terpenoids; the most structurally varied class of plant natural products. The name terpenoid, or terpene, derives from the fact that the first members of the class were isolated from turpentine (“terpentin” in German). All terpenoids are derived from repetitive fusion of branched five-carbon units based on an isopentane skeleton. These monomers generally are referred to as isoprene units because thermal decomposition yields the alkene gas isoprene as a product. For these reasons the

terpenoids are often called isoprenoids.

HORT640: Lecture 21, Week 11

Lecture title: Overview of N and S assimilation pathways and amino acid biosynthesis (Rhodes)

Date: November 3, 2016

Objectives: This Lecture provides a brief introduction to the pathways of nitrate and sulfate reduction, and the major pathways of synthesis of amino acids. Metabolic interfaces to central carbon metabolism will be revisited. Subsequent Lectures will take a closer look at individual pathways and their regulation.

HORT640: Lecture 22, Week 12

Lecture title: Nitrate uptake and reduction (Rhodes)

Date: November 8, 2016

Objectives: This Lecture will focus on the nitrate uptake systems in plants, and the regulation of nitrate reduction to ammonia. Particular attention is given to the regulation of nitrate reductase.

HORT640: Lecture 23, Week 12

Lecture title: Ammonia assimilation and recycling (Rhodes)

Date: November 10, 2016

Objectives: This Lecture focuses on the pathways of ammonia assimilation in plants. There is strong genetic and biochemical evidence that the main pathway of ammonia assimilation involves the glutamine synthetase-glutamate synthase cycle. Glutamate dehydrogenase primarily functions in glutamate catabolism in plants.

HORT640: Lecture 24, Week 13

Lecture title: Glycine and serine synthesis and aminotransferase reactions (Rhodes)

Date: November 15, 2016

Objectives: This Lecture will focus on the interconversions of glycine and serine, with emphasis on the glycine decarboxylase complex, serine transhydroxymethylase, and the involvement of tetrahydrofolate in these reactions. This series of reactions interfaces with plant one-carbon (C1) metabolism involved in the synthesis of many secondary plant products. This Lecture will also focus on the role of aminotransferases in amino acid

biosynthesis. The central role of pyridoxal phosphate as a cofactor will be discussed.

Assignments: HORT640 Quiz 4.

HORT640: Lecture 25, Week 13

Lecture title: Branched-chain amino acid, threonine and lysine metabolism; Proline, arginine and ornithine synthesis; Polyamines and non-protein amino acids (Rhodes)

Date: November 17, 2016

Objectives: This Lecture focuses on the biosynthesis of the branched-chain amino acids (valine, leucine and isoleucine) and the aspartate family amino acids threonine and lysine. Isoleucine derives its carbon skeleton from threonine. This Lecture also provides an overview of proline, arginine and ornithine biosynthesis, and an introduction to some non-protein amino acids and polyamines derived from the arginine-ornithine cycle. The non-protein amino acids canavanine and canaline are arginine and ornithine analogs, respectively. They are accumulated by certain legumes and play a role in defense against insects.

HORT640: Lecture 26, Week 14

Lecture title: Sulfate assimilation; cysteine and methionine synthesis and metabolism (Rhodes)

Date: November 22, 2016

Objectives: This Lecture focuses on the uptake and metabolism of sulfate to the sulfur amino acids cysteine and methionine, and the various metabolic fates of these amino acids (e.g. glutathione, glucosinolates, S-adenosyl-methionine, SMM, ethylene)

Assignments: HORT640 Quiz 5.

November 24, 2016. No Class, Thanksgiving Vacation

HORT640: Lecture 27, Week 15

Lecture title: Histidine and aromatic (tyrosine, phenylalanine and tryptophan) amino acid synthesis (Widhalm)

Date: November 29, 2016

Objectives: This Lecture provides an overview of the pathways of synthesis of

histidine, phenylalanine, tyrosine and tryptophan in plants. An important plant product derived from the tryptophan pathway is the hormone, auxin (indole acetic acid). Phenylalanine, tyrosine and tryptophan are used in the synthesis of a number of secondary plant products.

HORT640: Lecture 28, Week 15

Lecture title: Secondary products derived from aromatic amino acids (Widhalm)

Date: December 1, 2016

Objectives: This Lecture provides an overview of secondary products derived from aromatic amino acids, including tryptophan alkaloids, glucosinolates, cyanogenic glucosides, flavonoids and lignin.

Assignments: HORT640 Quiz 6.

HORT640: Lectures 29 and 30, Week 16

Lecture title: Mini-symposium [student presentations] (Rhodes, Widhalm)

Date: December 6 and December 8, 2016

Assignments: Students will give oral presentations of their powerpoint slides.

The final assignment for the course is for you to select any aspect of plant carbon, nitrogen, sulfur or secondary metabolism of your choice, and develop a PowerPoint slide presentation around this subject. Your presentation should succinctly convey the current state of knowledge of the field that you have chosen, and highlight promising future research directions that may address unresolved questions.

You should discuss your choice of subject with David Rhodes or Josh Widhalm.

On the last week of class we will hold a mini-symposium in which students will present their slides [20 min per presentation], with 5-10 min for questions and discussion.

This assignment will be worth 100 points. Sixty (60) points will be awarded for slide quality, and 40 points for presentation during the mini-symposium.

Presentations will begin at 1:30 PM on December 6 and December 8, 2016.