

Biochemical and Structural Studies of Dicamba Monooxygenase and Development of Dicamba-Resistant Crops

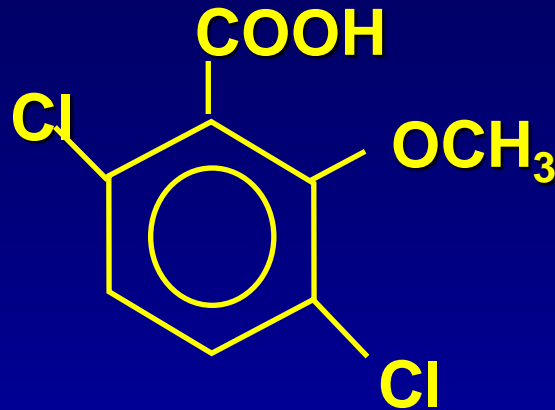
Don Weeks

**Department of Biochemistry
University of Nebraska - Lincoln**

**Workshop on Grapes and Herbicides
November 5, 2016, 2015**



Dicamba



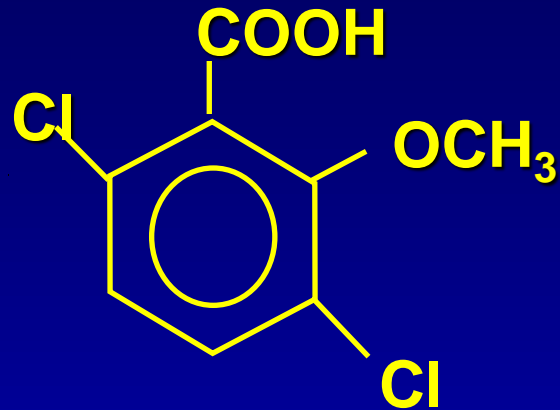
An Auxin-type Herbicide

Mimics the plant hormone, Indoleacetic Acid (IAA)

Too Much Hormone Kills Plants

**Broadleaf (Dicot) Plants are More Sensitive Than
Grassy (Monocot) plants**

Dicamba



Uses:

Dicamba is used to control broadleaf weeds (dicots) in grass-type crops (monocots). Until now, all broadleaf crops were sensitive to treatment with dicamba.

Goal:

Convert sensitive broadleaf crops (soybeans, cotton, vegetables) into dicamba-tolerant crops.

Strategy:

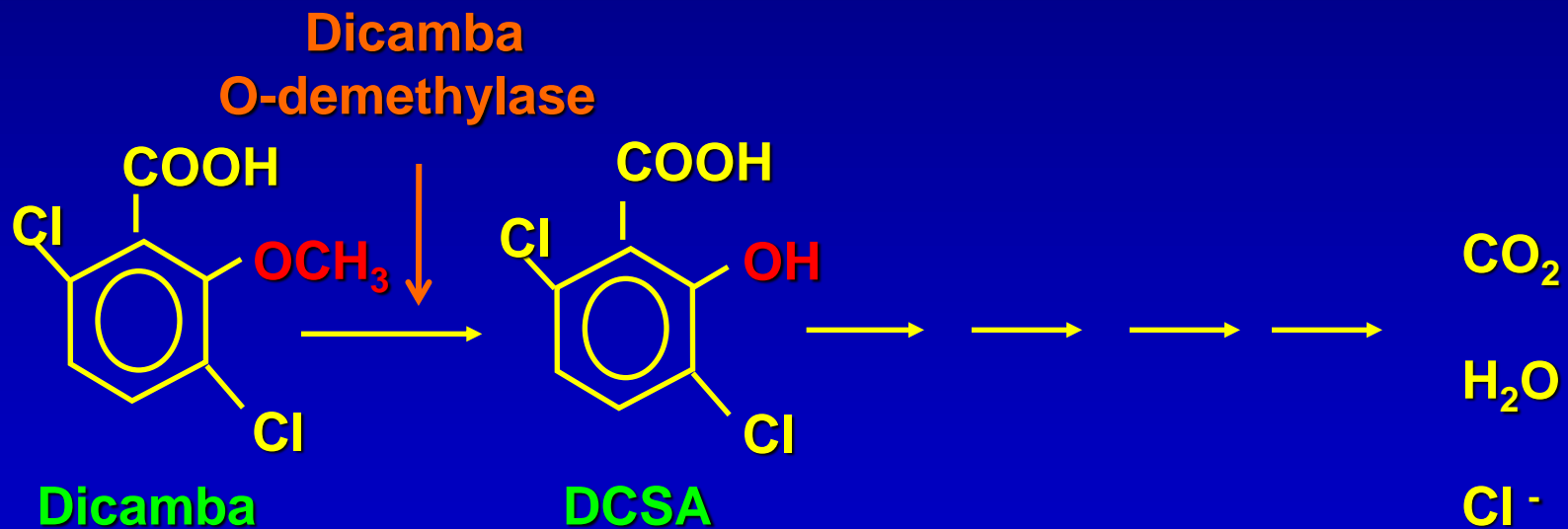
Place a gene in sensitive crops that produces an enzyme capable of inactivating dicamba.

Strategy:

1. Find bacterium with dicamba degrading enzyme
2. Purify the enzyme to homogeneity
3. Obtain N-terminal amino acid sequence
4. Design “degenerate” oligonucleotide probes
5. Use probes to select target gene
6. Genetically engineer gene to be a plant gene
7. Produce transgenic plants expressing the gene
8. Spray plants with dicamba - Hope for the best

Dicamba Degrading Bacterium: *Stenotrophomonas maltophilia*, strain D16

- Utilizes Dicamba as a Sole Carbon and Energy Source



- First Step in Degradation Inactivates Dicamba

But wait!

Life is not so simple!

Dicamba O-demethylase is not a single enzyme

It is 3 separate enzymes working together in a chain reaction:

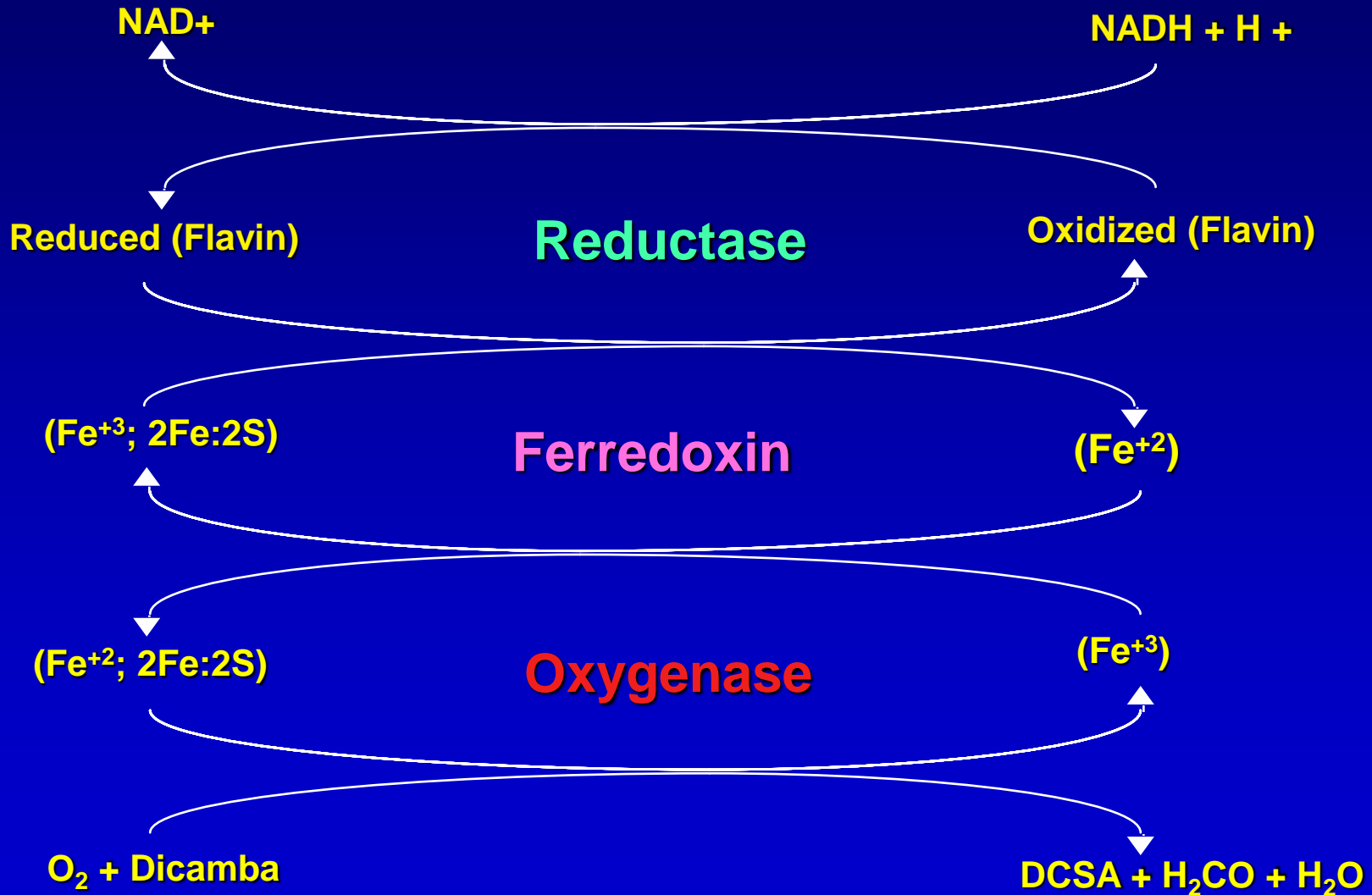
A reductase

A ferredoxin

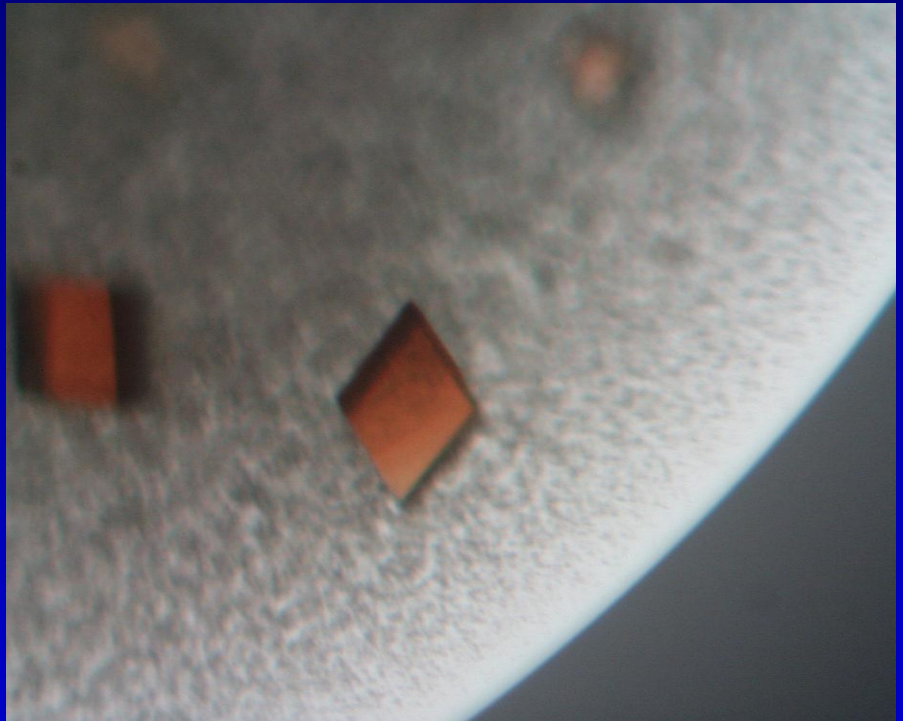
A monooxygenase

Dicamba Monooxygenase (DMO)

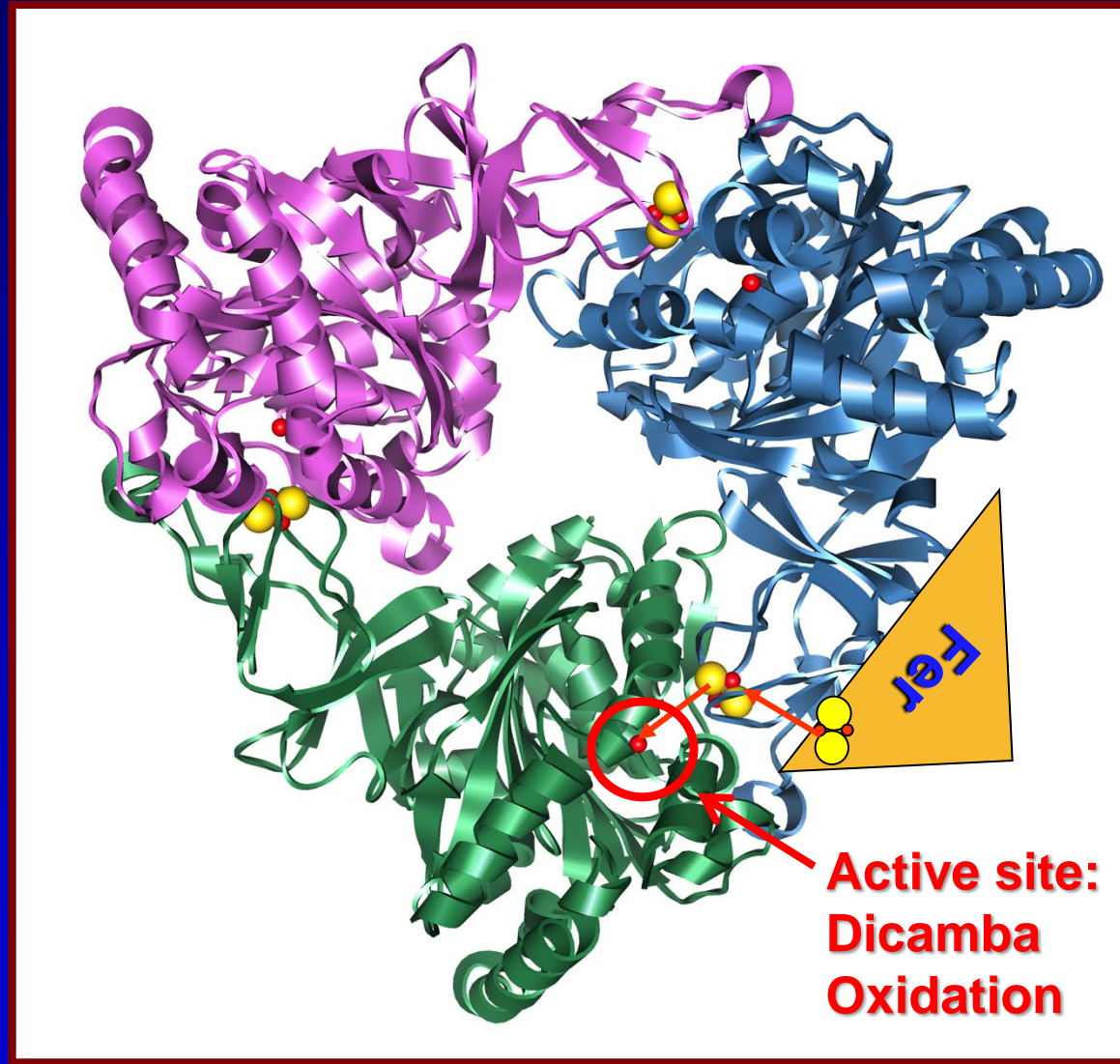
Dicamba O-Demethylase is a Three Component Enzyme System



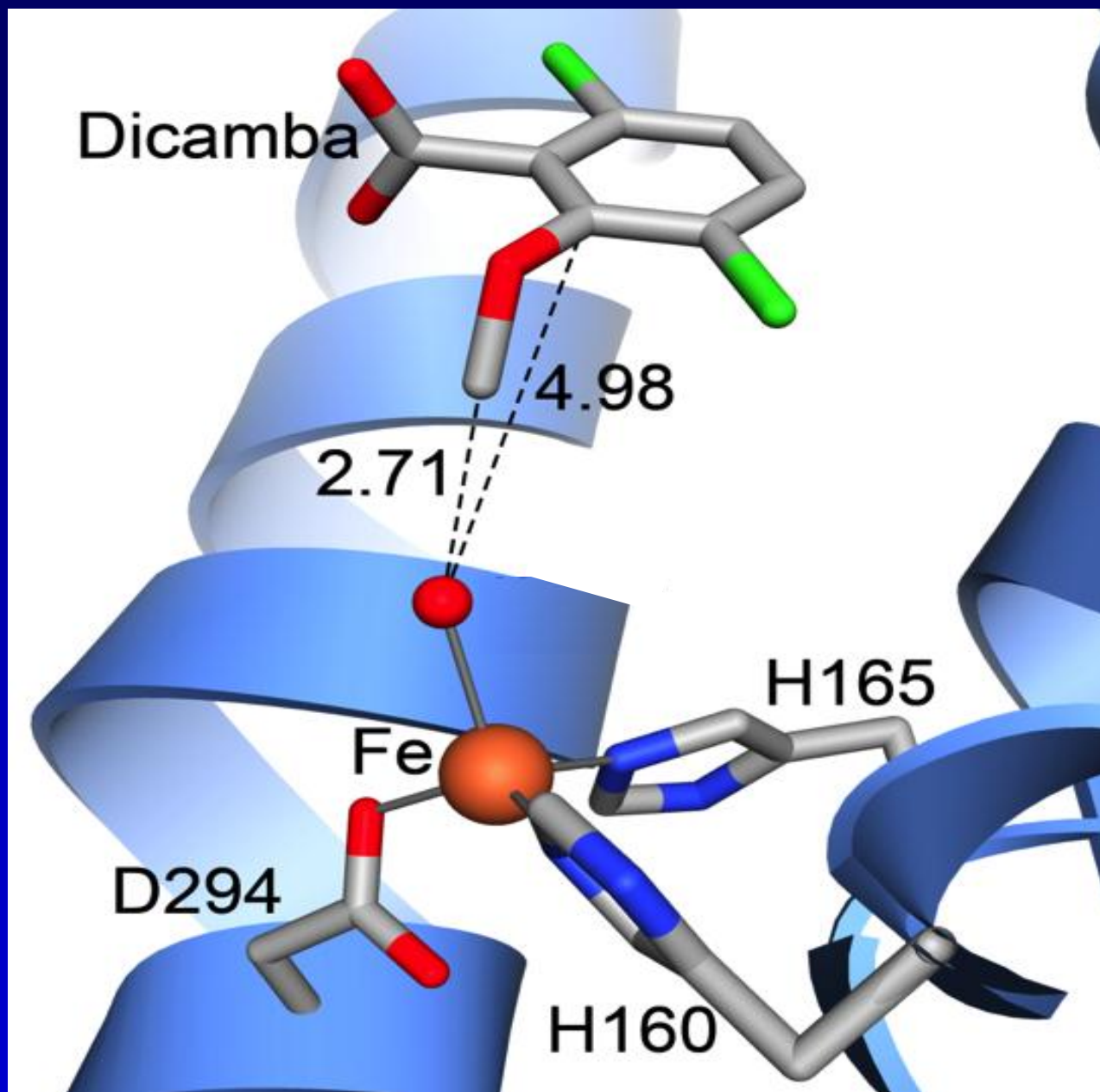
Crystals of DMO



Dicamba Monooxygenase



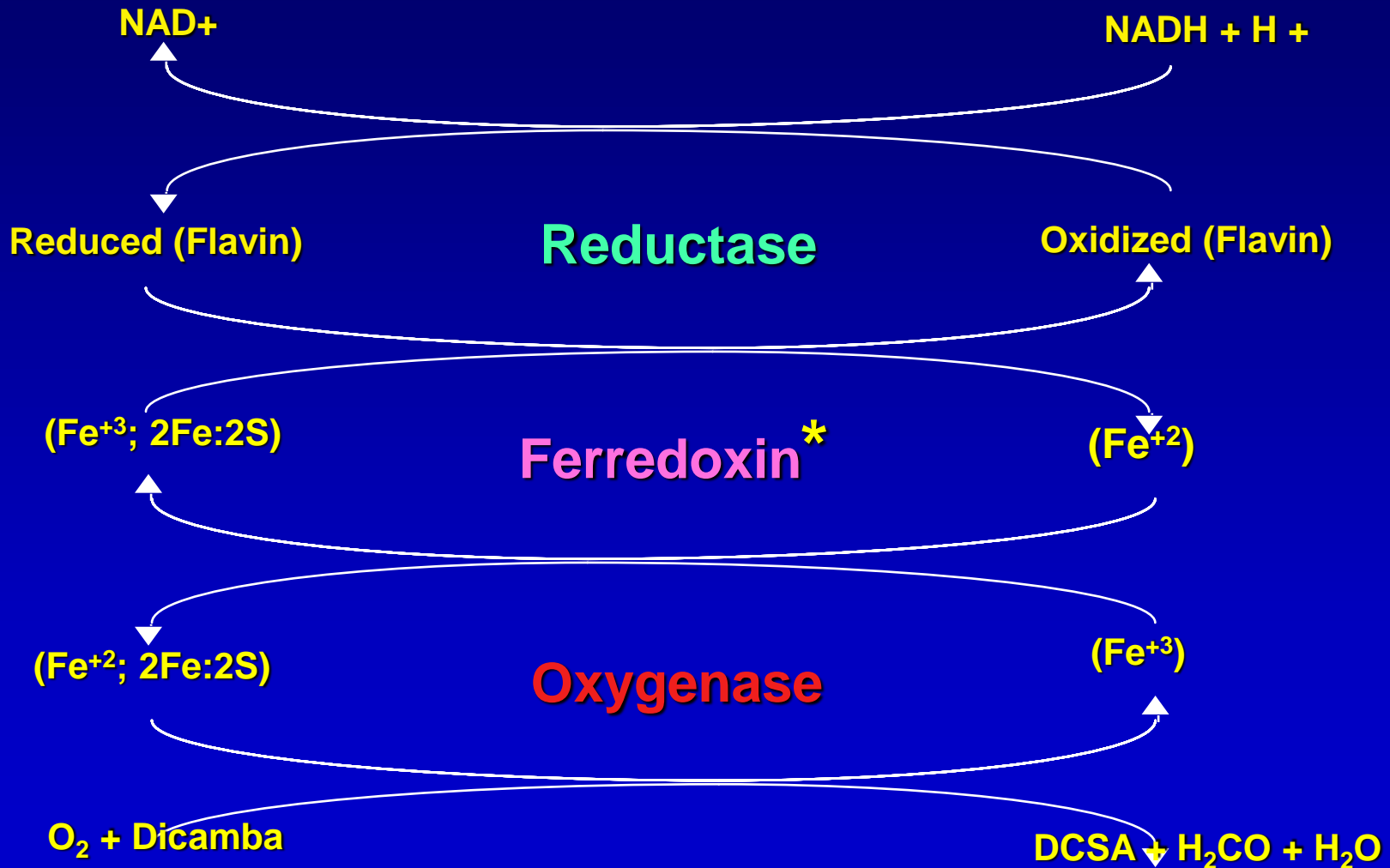
Dicamba Oxidation Reaction



Can DMO Provide Dicamba Resistance in Transgenic Plants?

**Genetic Engineering of the
Bacterial DMO Gene
into a Plant DMO Gene**

Dicamba O-Demethylase is a Three Component Enzyme System



*Bacterial Ferredoxin ~ Chloroplast Ferredoxin

Target DMO to the Chloroplast

The Bacterial Ferredoxin is Quite Similar in Structure to Plant Chloroplast Ferredoxin

Target DMO to the Chloroplast where there is an Abundance of Ferredoxin

If Successful, Only the Single DMO Gene will be Needed in Transgenic Plants

Concentration-dependent Sensitivity to Dicamba



0

0.0156

0.031

0.062

0.125

0.25

0.5

Conc. of Dicamba (lb/acre)

Recommended Rates

Test for Dicamba Resistance

Front view



Transgenic

Control

Top view



Dicamba @ 5.6 kg/ha (5.0 lb/A)

10-20 X Recommended Rate

Decreased Sensitivity to Dicamba in Tobacco

Wild-type Plants

Sensitive to 0.001 lb/acre

Nuclear Genome Transformants

Resistant to >25 lb/acre

Chloroplast Genome Transformants

Resistant to >50 lb/acre

> 25,000X Decrease in Sensitivity

Soybean Field Trials Summer 2005



**Dicamba Resistant
Soybean Plants**

**Nontransgenic
Soybean Plants**

**Dicamba resistance gene technology licensed to Monsanto
Important in controlling tough glyphosate-resistant weeds**

Glyphosate-resistant Weeds

- A major Economic Problem



Soybean Field with Glyphosate-resistant Marehail

Dicamba Kills Most Glyphosate-resistant Broadleaf Weeds

Dicamba-Tolerant Soybeans Expected to Provide the Most Effective Weed Management System

2009 Southern Illinois University Trials of Xtend soybean varieties



Non-treated
Control



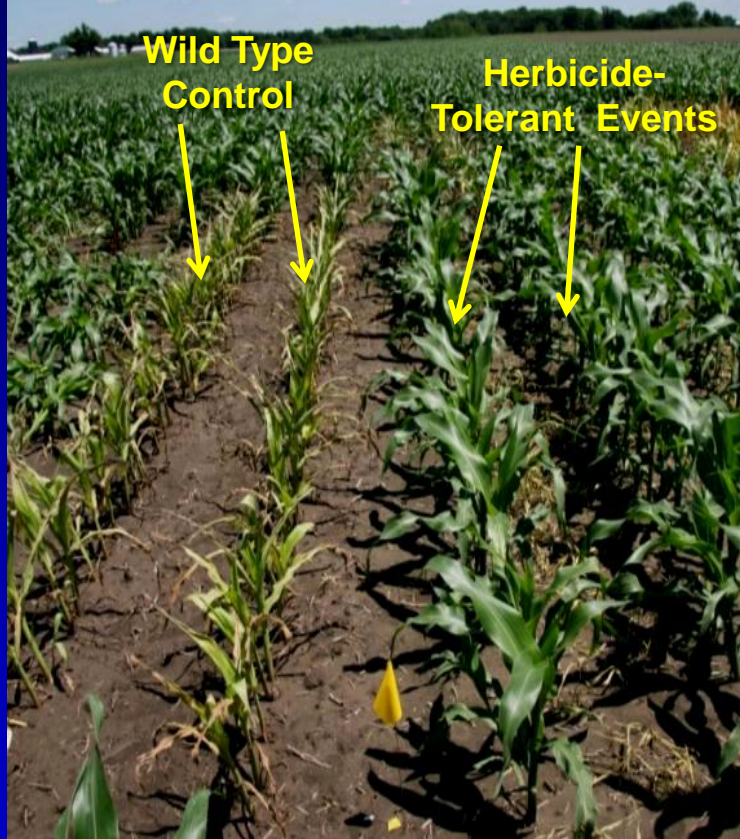
Dicamba mixed with *Roundup* on
RR2Y/dicamba-tolerant soybeans

- Roundup plus dicamba provides excellent control of hard to control weeds and glyphosate-tolerant weeds

Commercialization Began In 2016 **Monsanto**

Three Herbicide Options Would Expand Weed Control Choices For Farmers

2009 U.S. Field Trials Jerseyville, Illinois – June 2009



Wild Type vs. Herbicide Tolerant Event
Glyphosate 3 lbs. plus Dicamba 1 lb. @ V7



Wild Type vs. Herbicide Tolerant Event
Glufosinate 1.28 lb. @ V7

- Multiple modes of action expand grower choice and efficacy
- Cost effective resistance management
- Grower efficiency through wider application window

Monsanto

Dicamba-resistant Crops in Development

Canola

Wheat

Sugar Beet

Alfalfa

Engenia:

A lower volatility dicamba formulation

Monsanto

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Thank You
Questions?
