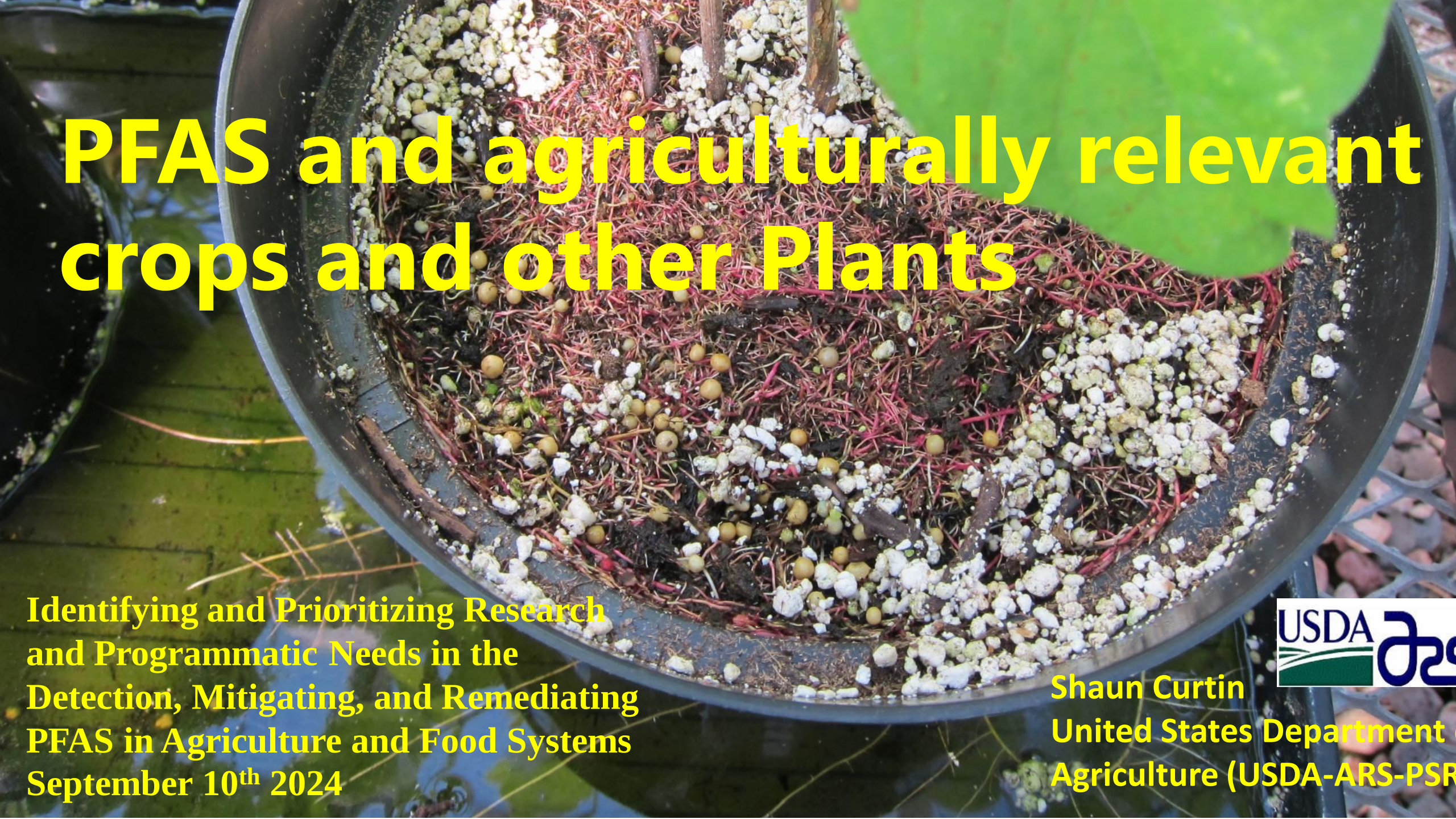
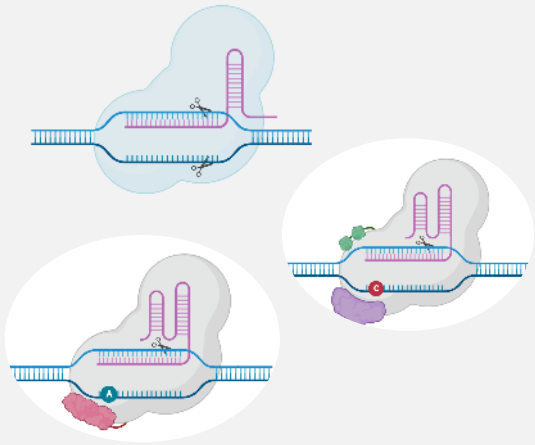
PFAS and agriculturally relevant crops and other Plants



Identifying and Prioritizing Research and Programmatic Needs in the Detection, Mitigating, and Remediating PFAS in Agriculture and Food Systems
September 10th 2024

Shaun Curtin
United States Department
Agriculture (USDA-ARS-PSR

Reagents



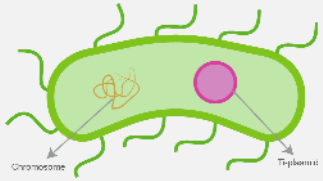
Targeted mutagenesis
- *Single genes*
- *Multiple genes*
- *QTLs*

Base edits
- Amino acid changes
- miRNA target disruption

Targeted knock-in

Prime edit

Transformation



Whole & hairy root txn
Selectable markers
Developmental regulators (DRs)
Agrobacterium
- GAANTY
- Shooty-Agro
- Shooty-rooty-Agro

Legumes

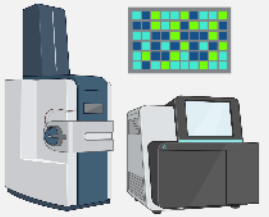
Soybean

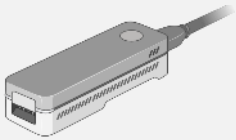
Medicago **Alfalfa**

Lentil

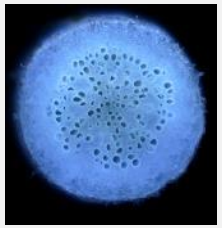
Gastrolobium spp.

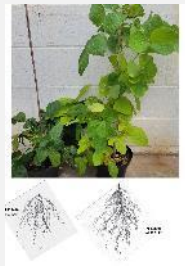
Genotyping

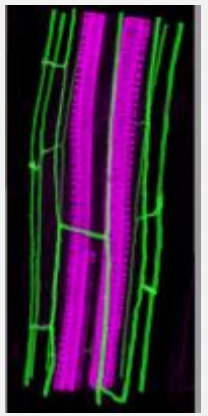




Phenotyping







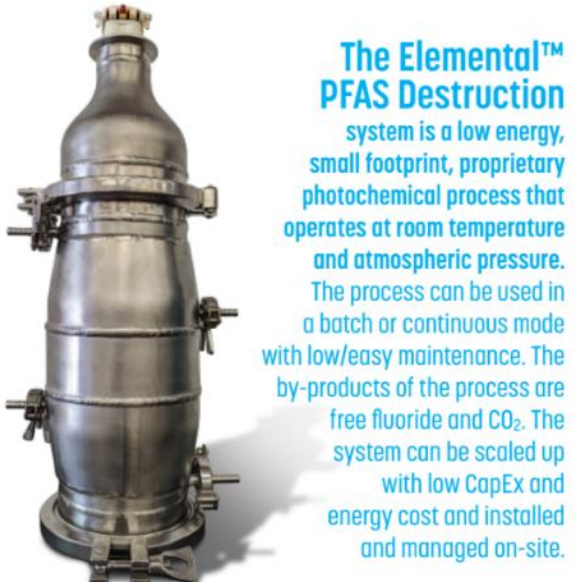
PFAS Background

- PFAS, a large group of chemicals known as per- and polyfluoroalkyl substances.
- Man-made compounds with wide-spread commercial use
- Highly stable carbon–fluorine (C–F) bond
- Environmental persistence, toxicity and bioaccumulation
- Remediation technologies to remove these chemicals

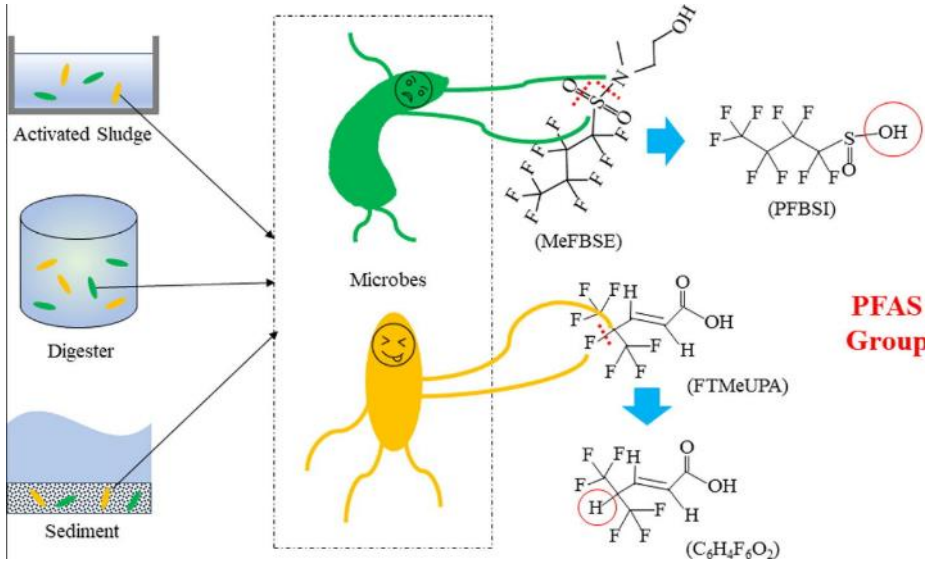
Common remediation technologies

Excavation/removal
Physical/chemical treatment
Thermal treatment
Biological treatment

Bioventing
Biosparging
Bioaugmentation
Phytoremediation

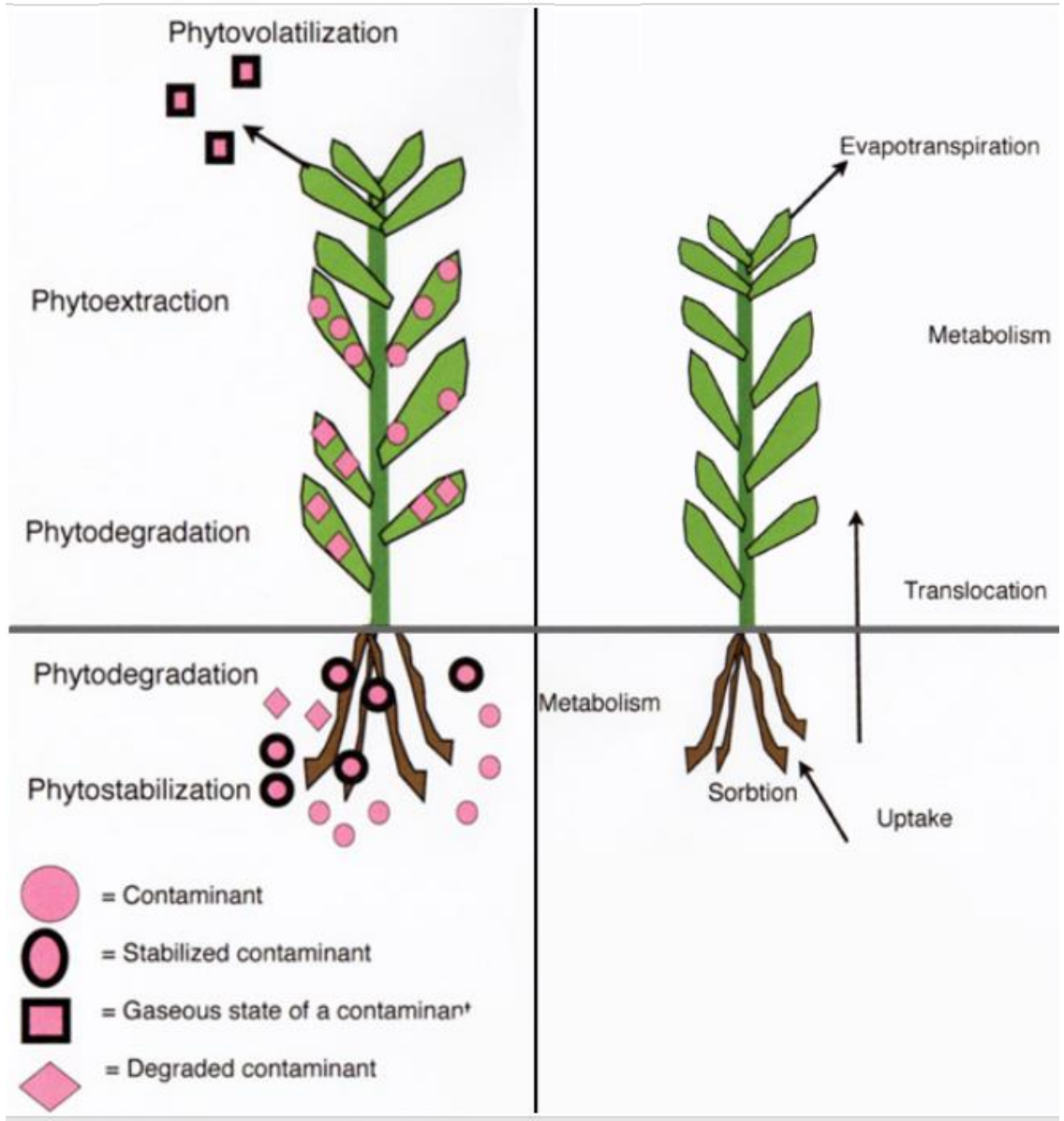


https://clarostechnologies.com/



Zhang et al 2022 Biodegradation of per- and polyfluoroalkyl substances (PFAS): A review

Phyto-remediation technologies



Phytoextraction – accumulation of contaminant from at is removal by plant harvest

Phytostabilization - contaminants are retained in the soil

Phytodegradation - organic contaminants are converted to less harmful substances

Phytovolatilization - contaminants are converted to a gaseous state and released into the atmosphere

Greipsson, S. (2011) Phytoremediation. Nature Education Knowledge 3(10):7

Examples of phytoremediation

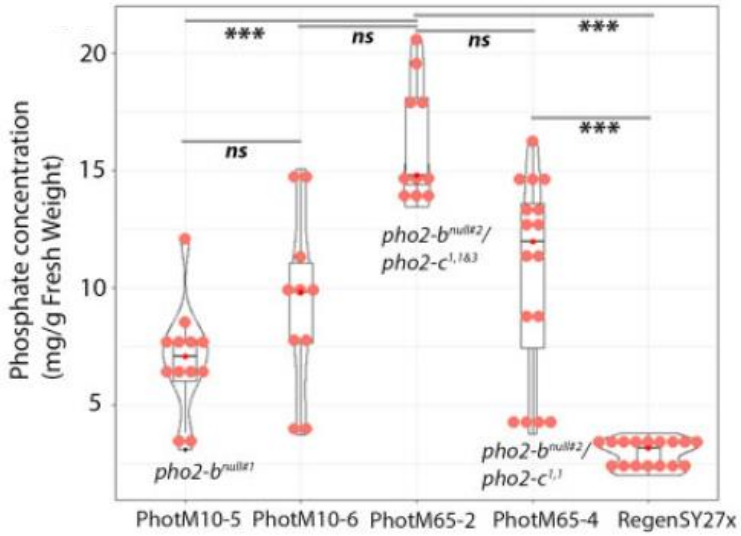
Repeated applications lead to P contaminated soil.

Eroded into waterways, damaging water quality

P is a finite mineral resource

The phenotype of 4x KO single *pho2-1* mutant plants

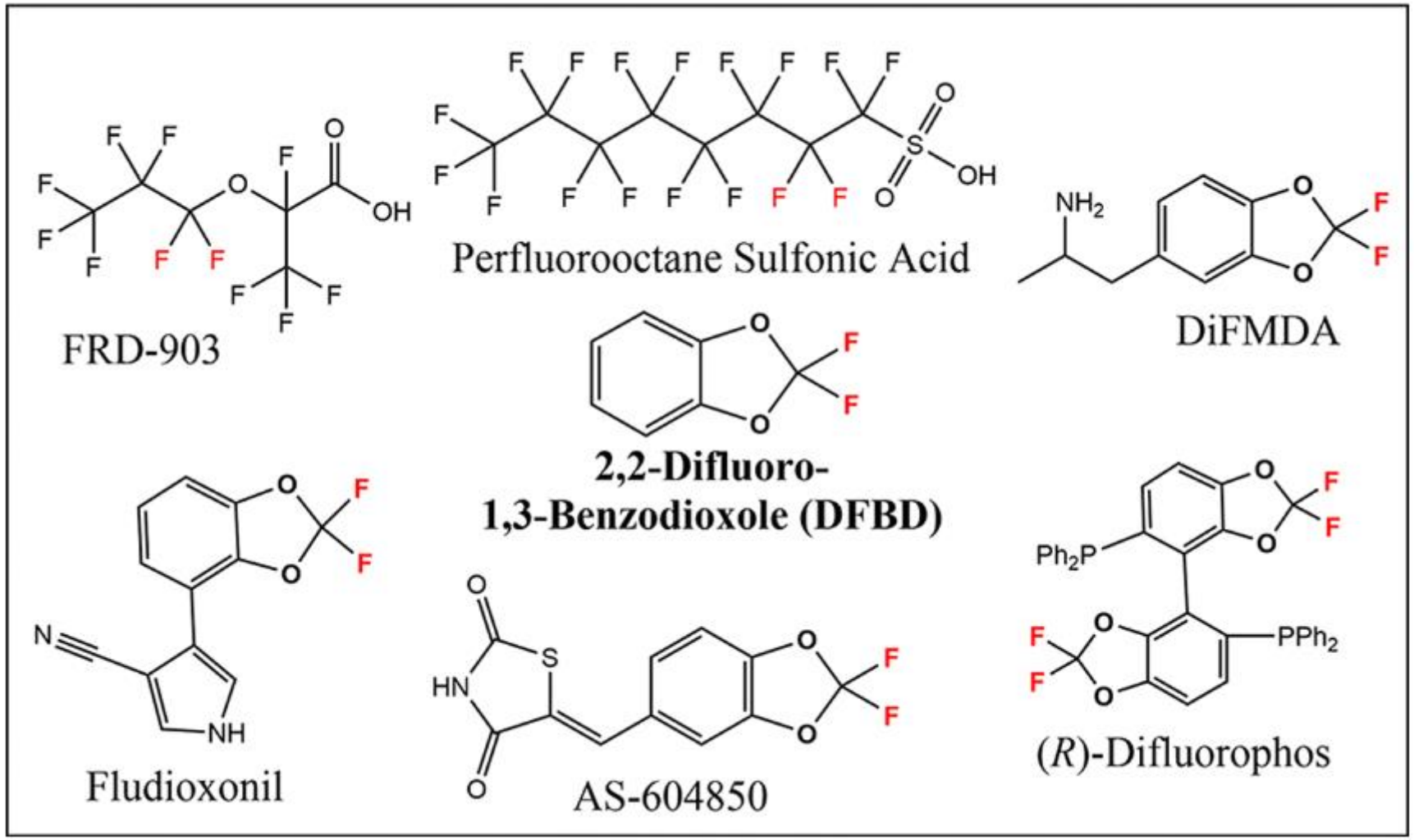
Two genes Pho2-B & Pho2-C, 4x haplotype copies. (Pho2-B1, Pho2-B2, Pho2-B3 & Pho2-B4)



10x fold increase in P_i observed in the octuple mutant

Miller et al 2022 Alfalfa (Medicago sativa L.) pho2 mutant plants hyperaccumulate phosphate

Examples of commercially relevant componds



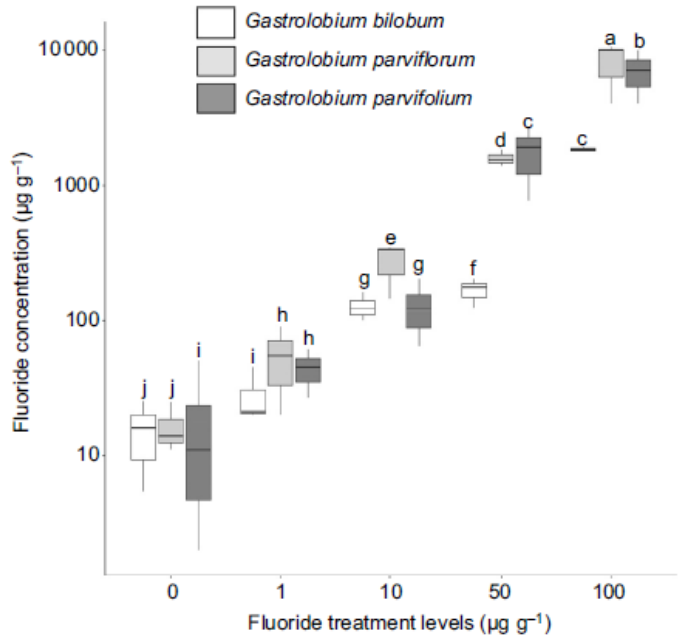
Gastrolobium spp. can accumulate extremely high levels of F^-

Gastrolobium parvifolium **aka 'Berry Poison"**

Gastrolobium cuneatum **aka 'River Poison'**

Gastrolobium bilobum **aka 'Heart Leaf Poison'**

Gastrolobium laytonii **aka 'Breelya' ' Kite-leaf Poision'**



Abubakari et al 2021 Fluoride hyperaccumulation in Gastrolobium species (Fabaceae) from Western Australia

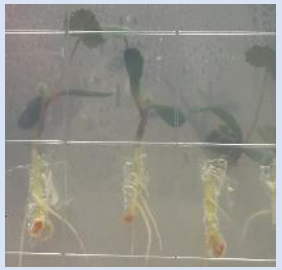
Hairy-root assay for generating transgenic roots

Use *Agrobacterium* hairy-root strains to rapidly test candidate dehalogenase and hydrolase activities

Ruby visual marker construct

A. rhizogenes used for T-DNA delivery

Seeds are germinated, root is removed and inoculated with the Agrobacterium

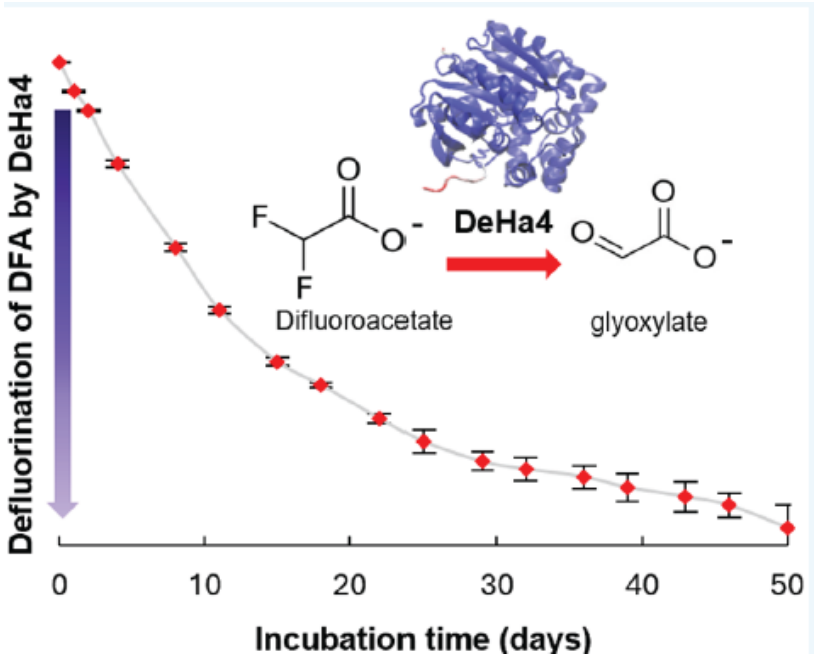


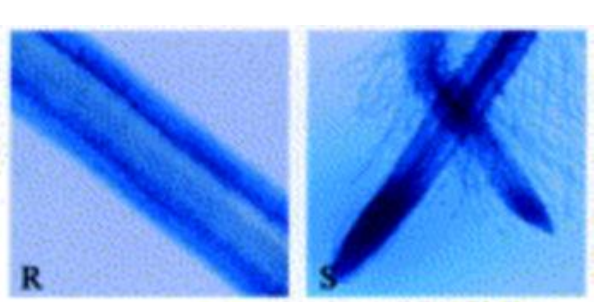
Two weeks after transformation

Six weeks after transformation Ruby-red is expressed in transgenic root tissue.

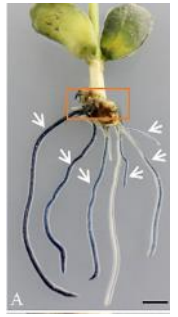
Screening promoter & candidate enzymes in plants

- Identify promoters with strong root expression
- Identify candidate defluorination enzymes

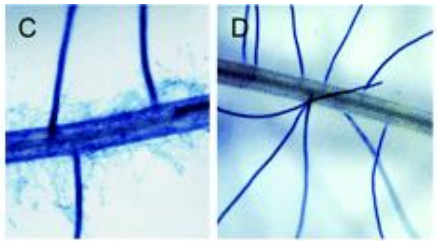




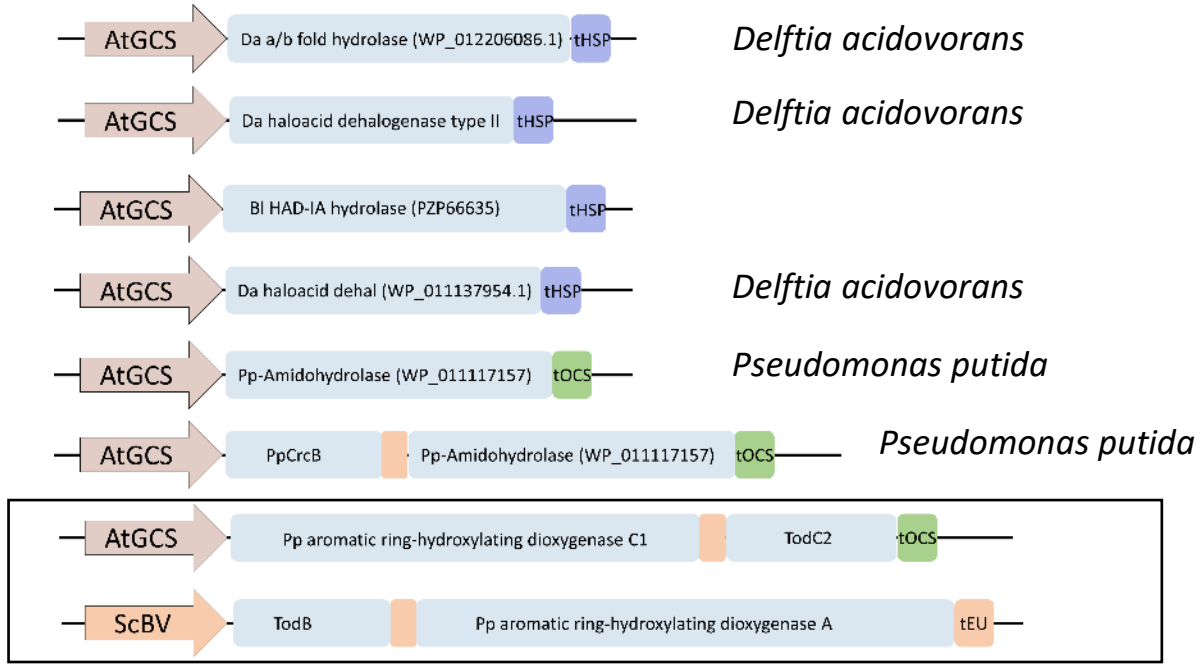
AtPyk10



AtGCSp



HvPht1

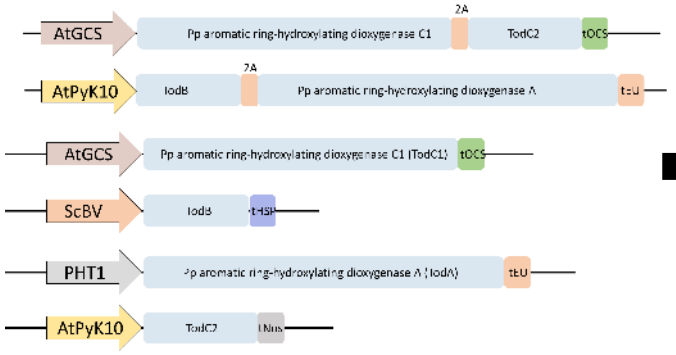


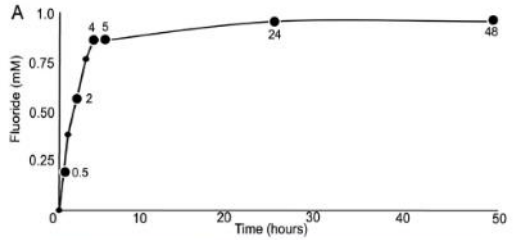
Liu et al 2022 AtGCS promoter-driven clustered regularly interspaced short palindromic

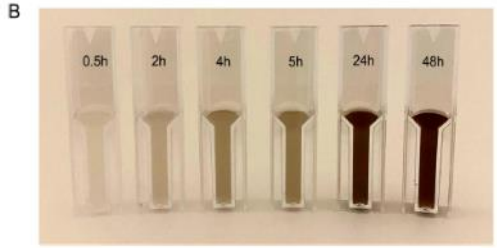
Nitz et al 2001 Pyk10, a seedling and root specific gene and promoter from Arabidopsis thaliana

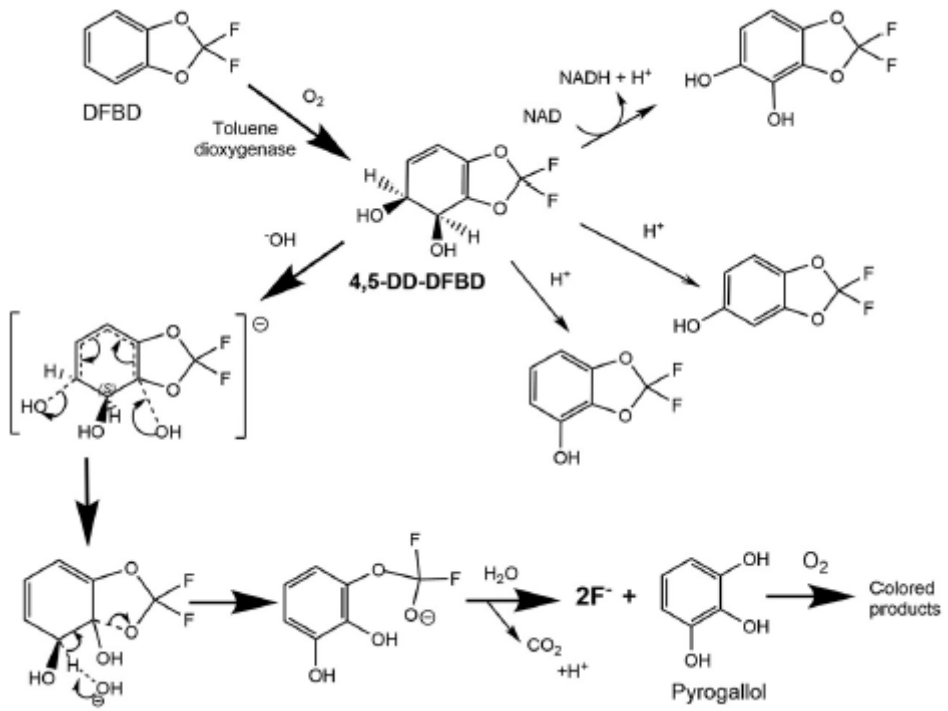
Farajollahi et al 2024 ACS Omega 2024, 9, 28546−28555

Demonstrating and detecting defluorination









Bygd et al 2021 Unexpected Mechanism of Biodegradation and Defluorination of 2,2-Difluoro-1,3-Benzodioxole by Pseudomonas putida F1

Questions to be answered

- Can *Gastrolobium* spp. be transformed by Agrobacterium?
- Commonly used PFOA (perfluorooctanoic acid) and PFOS (perfluorooctanoic sulfonic acid) or difluoracetate acid?
- How do we monitor the reaction, how do we know active defluorination is happening?

Acknowledgments

- Roy Scott
- Jack Okamuro
- Deb Samac
- Larry Wackett
- Thomas Niehaus