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The webinar will begin promptly at  
12:00 pm ET, 9:00 am PT



# SERDP and ESTCP Webinar Series

***The webinar will begin promptly at 12:00 pm ET,  
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- You have two options for accessing the webinar
  1. Listen to the broadcast audio if your computer is equipped with speakers
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Required conference ID: 6102000
- For any question or issues, please email [serdp-estcp@noblis.org](mailto:serdp-estcp@noblis.org) or call 571-372-6565

## Management of Energetic and Propellant Material Releases on Testing and Training Ranges

July 27, 2017



## Welcome and Introductions

Jennifer Nyman, Ph.D., P.E.  
Webinar Facilitator



# Webinar Agenda

- **Webinar Logistics** (5 minutes)  
**Dr. Jennifer Nyman**, Geosyntec Consultants
- **Overview of SERDP and ESTCP** (5 minutes)  
**Dr. Andrea Leeson**, SERDP and ESTCP
- **Overview of the Unique Environmental Challenges of Military Lands and Research Needs to Address these Challenges** (25 minutes + Q&A)  
**Dr. Elizabeth Ferguson**, U.S. Army Engineer Research and Development Center
- **Phytoremediation of Explosives Contaminated Soil by Transgenic Grass** (25 minutes + Q&A)  
**Dr. Neil Bruce**, University of York, United Kingdom
- **Final Q&A session**

# How to Ask Questions

Type and send questions at any time using the Q&A panel

Chat with Presenter:

Question|

Send

# In Case of Technical Difficulties

- Delays in the broadcast audio
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- Submit a question using the chat box

# SERDP and ESTCP Overview

Andrea Leeson, Ph.D.  
SERDP and ESTCP



# SERDP

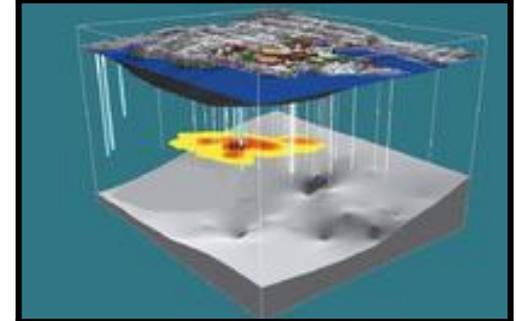
- Strategic Environmental Research and Development Program
- Established by Congress in FY 1991
  - DoD, DOE and EPA partnership
- SERDP is a requirements driven program which identifies high-priority environmental science and technology investment opportunities that address DoD requirements
  - Advanced technology development to address near term needs
  - Fundamental research to impact real world environmental management

# ESTCP

- Environmental Security Technology Certification Program
- Demonstrate innovative cost-effective environmental and energy technologies
  - Capitalize on past investments
  - Transition technology out of the lab
- Promote implementation
  - Facilitate regulatory acceptance

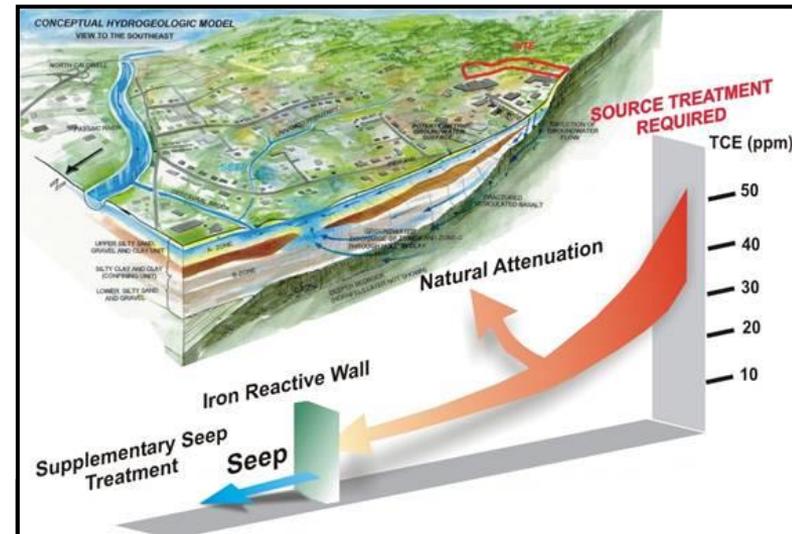
# Program Areas

1. Energy and Water
2. Environmental Restoration
3. Munitions Response
4. Resource Conservation and Resiliency
5. Weapons Systems and Platforms



# Environmental Restoration

- Major focus areas
  - Contaminated groundwater
  - Contaminants on ranges
  - Contaminated sediments
  - Wastewater treatment
  - Risk assessment



# SERDP and ESTCP Webinar Series

Date	Topic
August 17, 2017	Zinc Nickel Dip and Brush Plating
September 7, 2017	Research and Development Needs for Management of DoD's PFAS Contaminated Sites
September 21, 2017	Building Envelop Technologies
October 5, 2017	New Resource Conservation Insights to Desert Environments
November 2, 2017	Platforms for Underwater and Near-Shore Munitions Surveys

**For upcoming webinars, please visit**

<http://serdp-estcp.org/Tools-and-Training/Webinar-Series>



# SERDP • ESTCP SYMPOSIUM

2017 | Enhancing DoD's Mission Effectiveness

A three-day symposium showcasing the latest technologies that enhance DoD's mission through improved environmental and energy performance

- November 28 - November 30, 2017
- Washington Hilton Hotel  
1919 Connecticut Avenue, NW  
Washington, DC 20009
- ***Registration is open***

## Overview of the Unique Environmental Challenges of Military Lands and Research Needs to Address these Challenges

Dr. Elizabeth Ferguson  
United States Army Engineer  
Research and Development Center



# Agenda

- What makes military lands unique?
- Why do we care?
- What are the issues?
- What are the research gaps?



# Extent of DoD Lands

- 4,800 sites (excluding USACE lands)
  - 26.1 M acres worldwide (owned, leased, or occupied)
  - 11.6 M acres in the United States
    - Largest individual parcel – 2.3 M acres
- “Big four”: 3M acres in New Mexico, 2 M acres in California, 0.6M acres in Alaska, 0.5 M acres in Florida

*Sources: Federal Land Ownership: Overview and Data, Congressional Research Service, March 2017; U.S. DoD Office of the Deputy Under Secretary for Installations & Environment, Base Structure Report, Fiscal Year 2015 Baseline*

# Largest Army Training Ranges

	CONUS	OCONUS
Total ranges	441	57
Total area (M acres)	13.9	0.2
Average area (K acres)	31.6	3.3
Largest site	2.1 M acres, Land impact area, Land firing range, White Sands Missile Range, NM	39 K acres, Land maneuver, Land firing range, Hofenfels, Germany



CONUS – Contiguous United States  
 OCONUS – Outside continental United States

# DoD Environmental Program Priorities

- Protect the environment to ensure resources available for training and readiness
- Protect the health of military and civilian personnel living and working on bases
- Ensure DoD operations do not adversely impact surrounding communities
- Preserve resources for future generations



**Source:** Fiscal Year 2015 (FY15) Defense Environmental Programs Annual Report to Congress; DoD Instruction 4715.03 of March 18, 2011

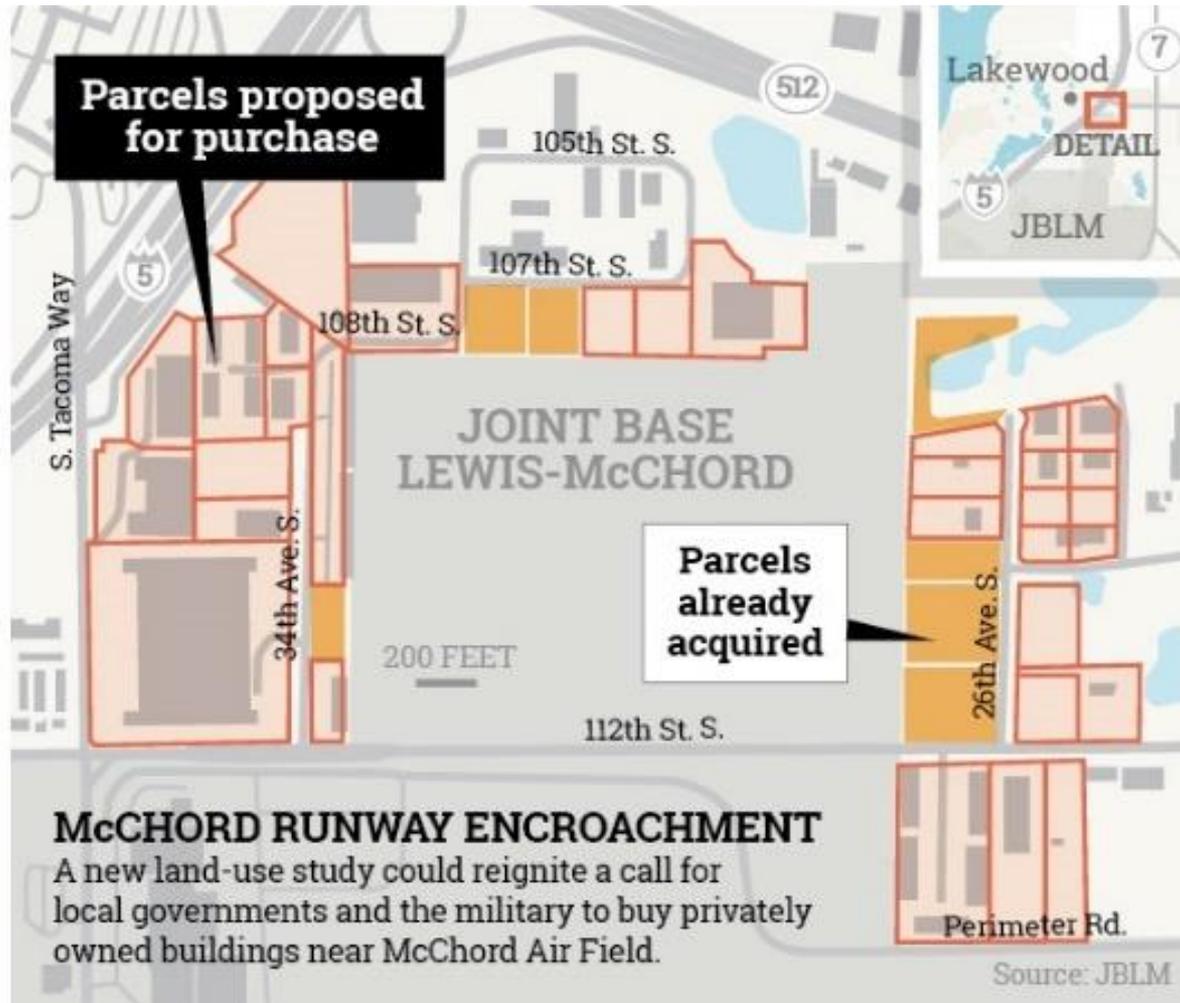
# Critical Environmental Issues for DoD

- Threatened and endangered species (TES)
- Munitions restrictions
- Spectrum operations
- Maritime sustainability
- Airspace
- Air quality
- Noise restrictions
- Adjacent land use
- Cultural resources
- Water quality/supply
- Wetlands
- Range transients
- Climate change





# Encroachment Issues (Continued)



# Novel Materials Impacting DoD Lands

- Insensitive munitions and new high explosive formulations
- Nano and other advanced materials
- In-situ manufacturing/additive materials
- Novel smokes and obscurants



# What Happens in a New Environment



# Some Research Gaps

- Extreme environments
  - Climate
  - Populations dynamic
  - Altered systems
- Novel materials/technologies
  - Impacts
  - Uses
- Assessment tools
- New TES management
- Sustainment techniques
- Remediation tools
- Computational simulation
- Environment as model



# Conclusions

- DoD has clear requirements and responsibilities as a federal landowner
- DoD uniquely impacts the lands for training purposes and the impacts are dynamic
- New environmental parameters will continue to challenge what we think we know
- Many, many research gaps remain



# *SERDP & ESTCP Webinar Series*

For additional information, please visit  
[https://serdp-estcp.org/Program-Areas/  
Environmental-Restoration/Contaminants-on-Ranges/](https://serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminants-on-Ranges/)

## **Speaker Contact Information**

elizabeth.a.ferguson@usace.army.mil; 601-634-4008



## Q&A Session 1



## Phytoremediation of Explosives Contaminated Soil by Transgenic Grass

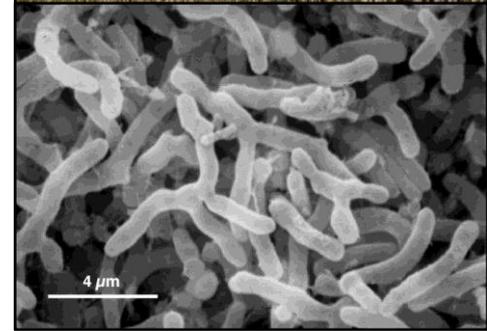
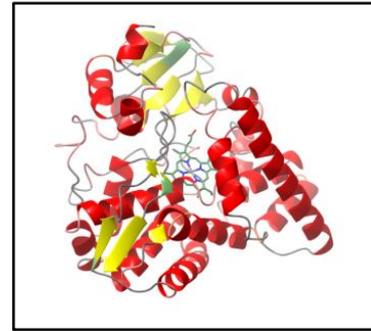


Dr. Neil Bruce  
University of York, United Kingdom



# Outline

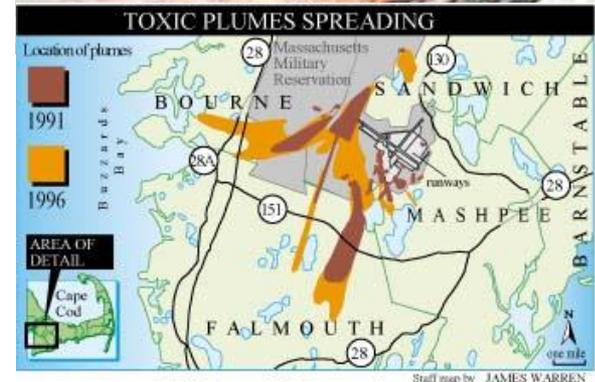
- Explosives pollution
- Phytotoxicity of explosives
- Microbial explosives degrading enzymes
- Engineering transgenic plants for the remediation of military ranges



# The Problem

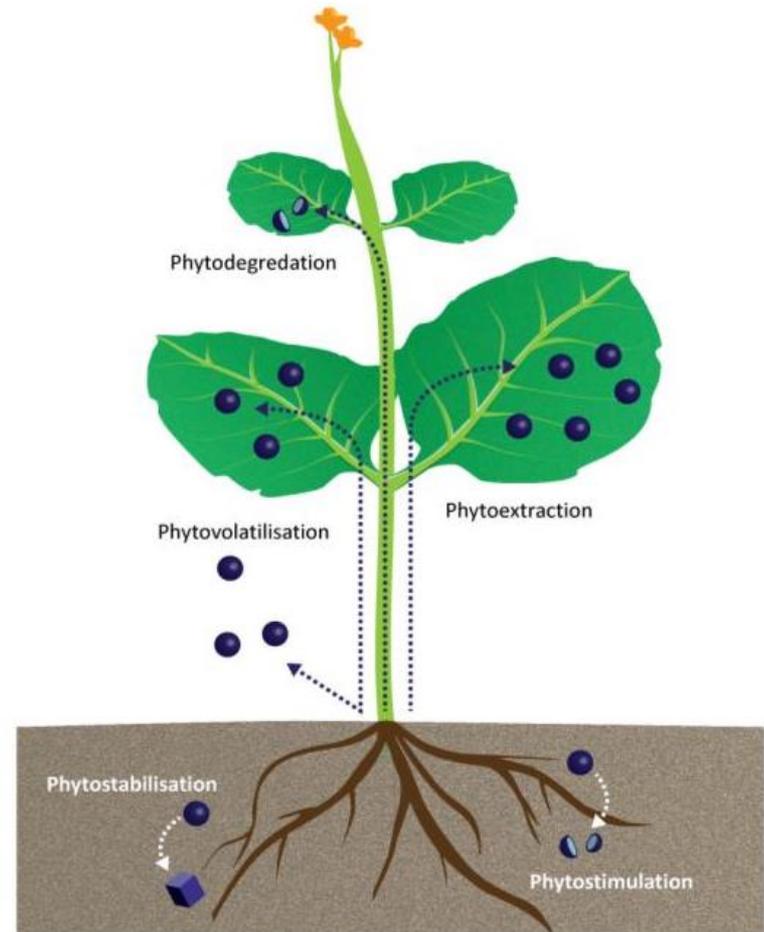
- RDX and TNT are toxic, and recalcitrant to degradation
- Large scale contamination of high explosives including manufacturing and storage facilities
- Over 10 million hectares contaminated by munition constituents on U.S. active ranges<sup>1</sup>
- Large amounts of groundwater are contaminated, e.g., Massachusetts Military Reservation

<sup>1</sup> U.S. General Accountability Office, *Department of Defense Operational Ranges, More Reliable Cleanup Cost Estimates and a Proactive Approach to Identifying Contamination Are Needed* (GAO Publication GAO-04-601, 2004; <http://www.gao.gov/products/GAO-04-601>)



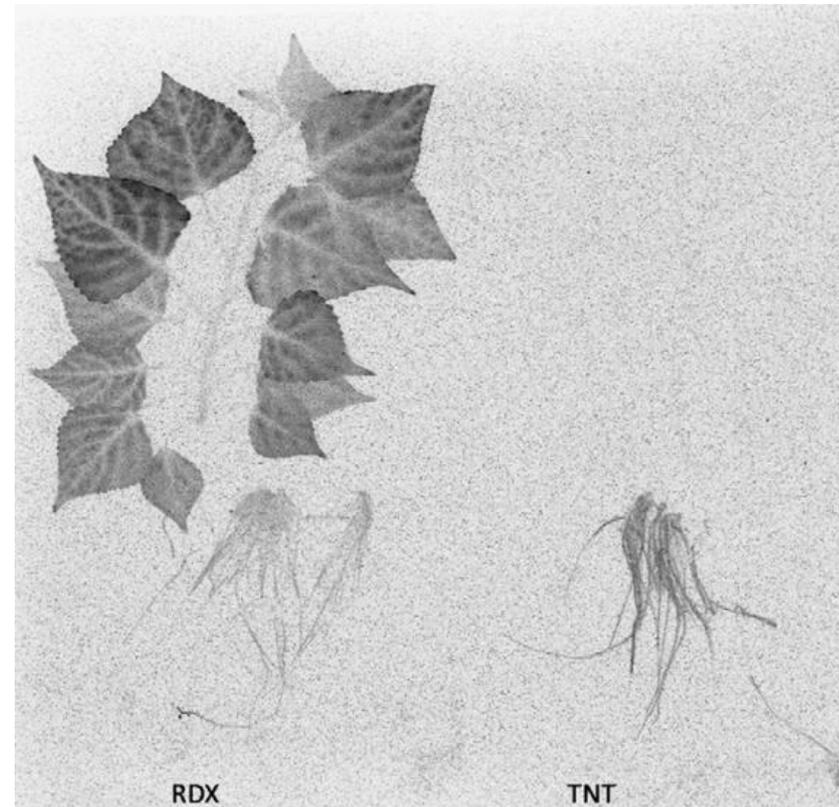
# Phytoremediation

- Low maintenance
- Minimally disruptive
- Cost-effective
- Aesthetically-pleasing
- Compatible with restoration ecology



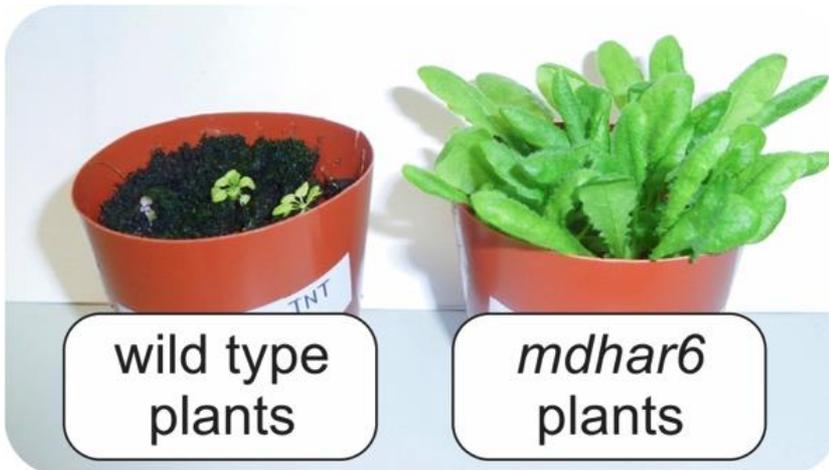
# Localization of TNT and RDX in plants

- Phosphor imager autoradiograph shows the distribution of [U-14C]-RDX and -TNT by poplars 48 h after exposure
- Plants are able to take up TNT and RDX
- Is it possible to engineer plants to improve their ability to break down or remove explosives from contaminated soil?



Courtesy of J. Schnoor

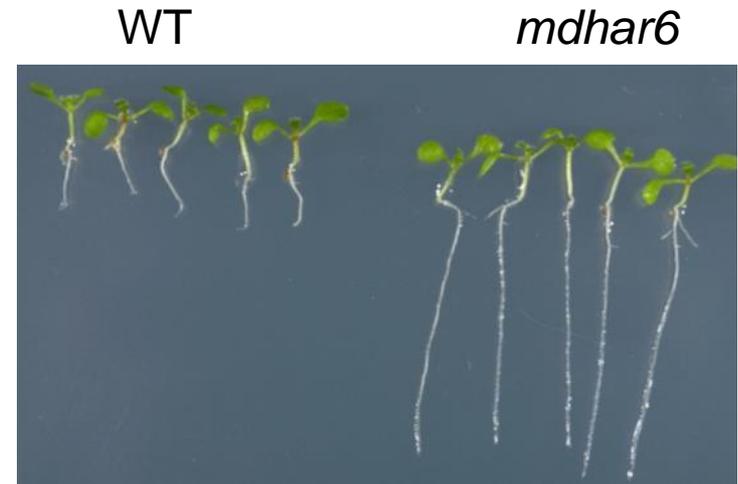
# Phytotoxicity of TNT



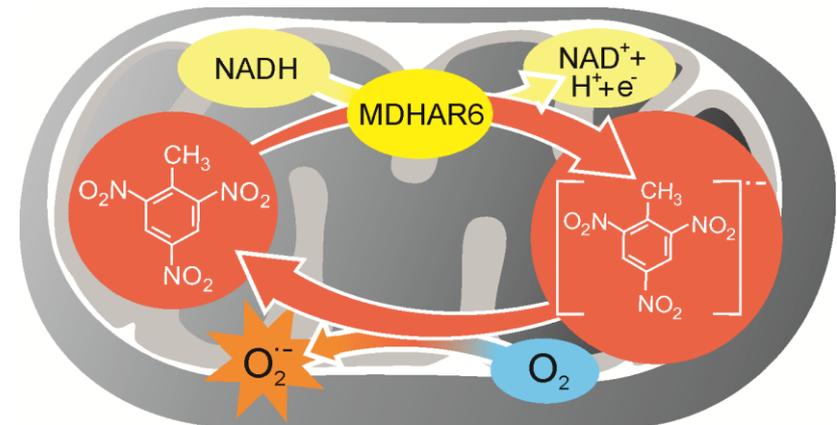
100 mg TNT/kg soil

- Monodehydroascorbate reductase 6 (MDHAR6) mediates TNT toxicity in plants.
- Produced new *mdhar6* plant lines with increased resistance to TNT toxicity

Johnston *et al.* 2015. *Science* 349: 1072

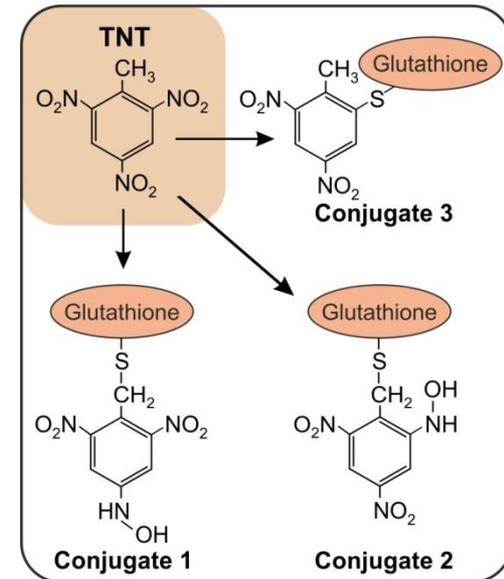
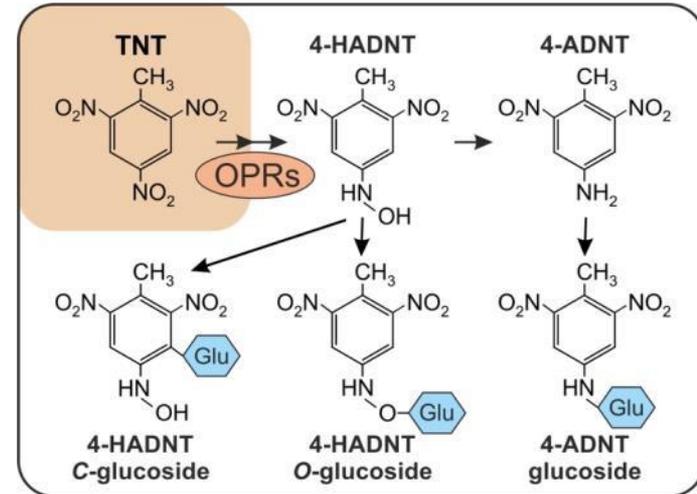
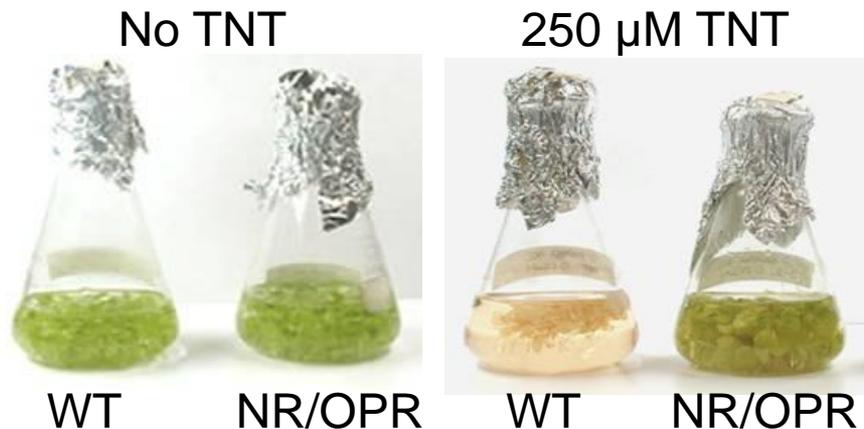


15  $\mu$ M TNT



# Plant Metabolism of TNT

- A fundamental understanding of TNT metabolism has allowed the development of improved TNT remediating plant lines



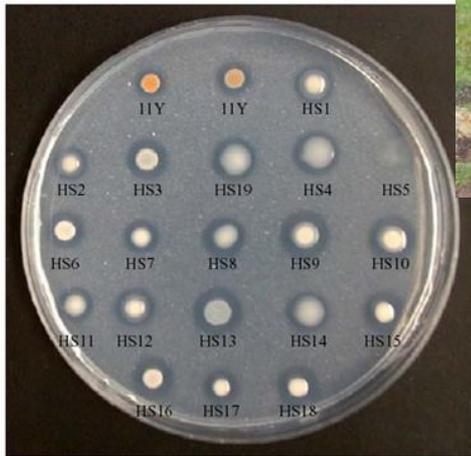
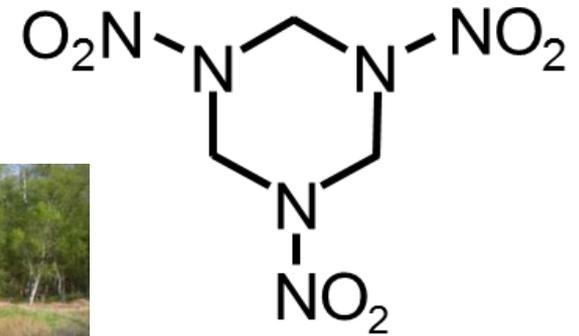
NR - Nitro reductase

OPR - Oxophytodienoate reductase

Tzafestas *et al.* 2017. *New Phytol* 214: 294; Gunning *et al.* 2014.

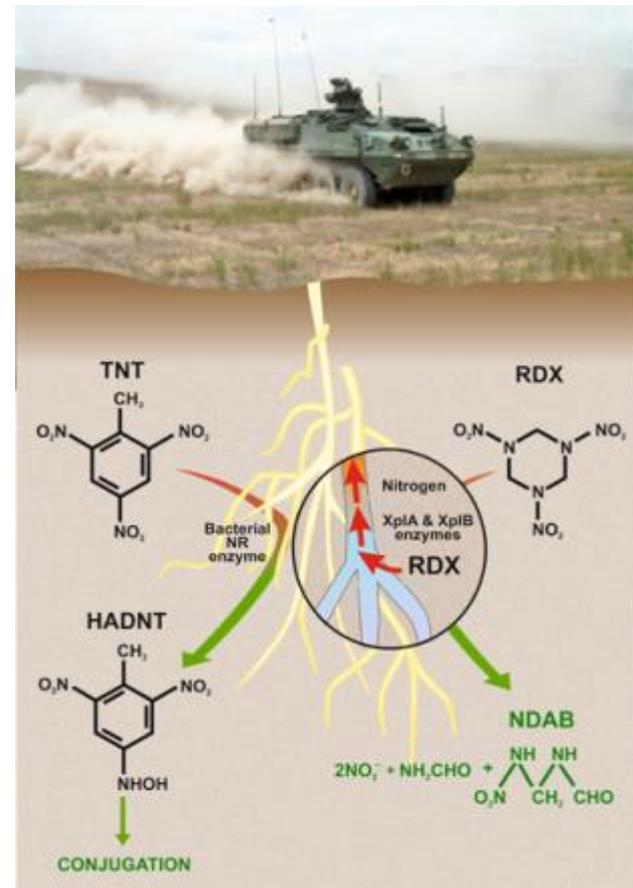
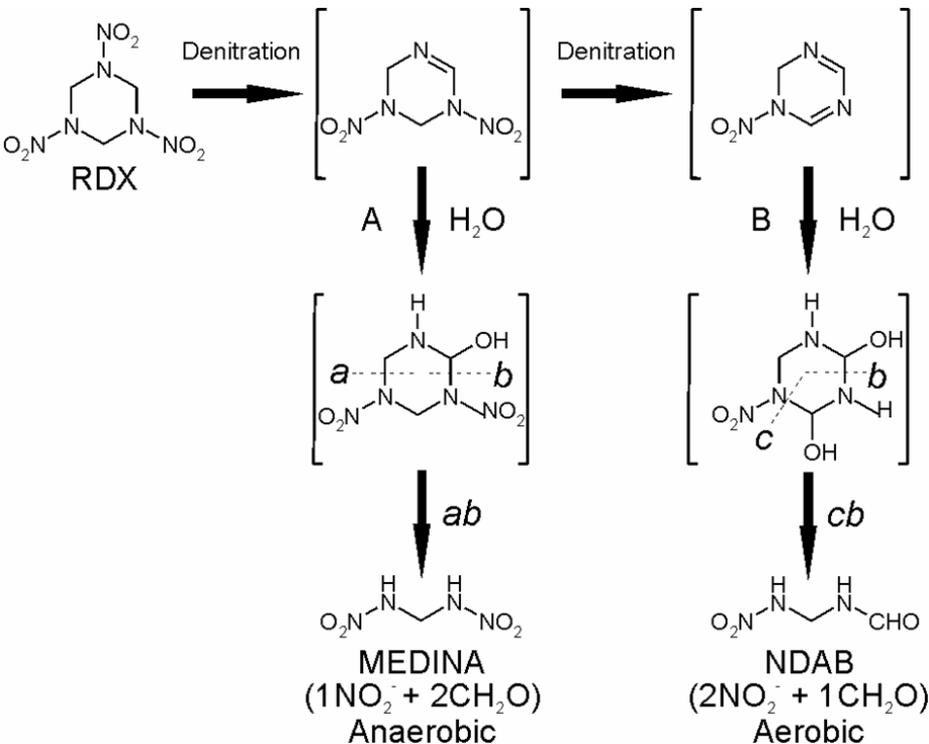
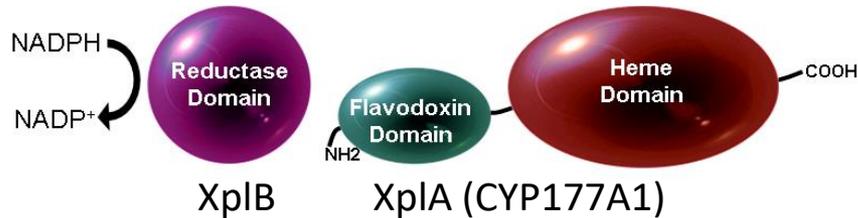
*Plant Physiol.* 165: 854; Rylott *et al.* 2011. *New Phytol.* 192: 405

# Isolating RDX Degrading Bacteria



Seth-Smith *et al.* 2008. *Appl. Environ. Microbiol.* 74: 4550  
 Chong *et al.* 2014. *Appl. Environ. Microbiol.* 80: 6601

# Engineering Plants for Phytoremediation of TNT and RDX

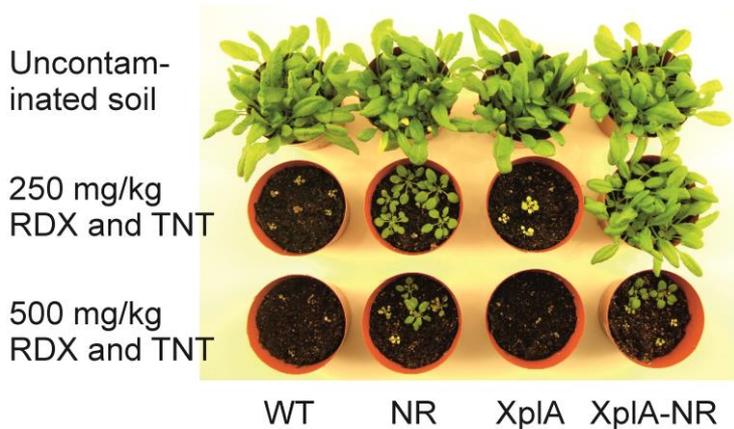
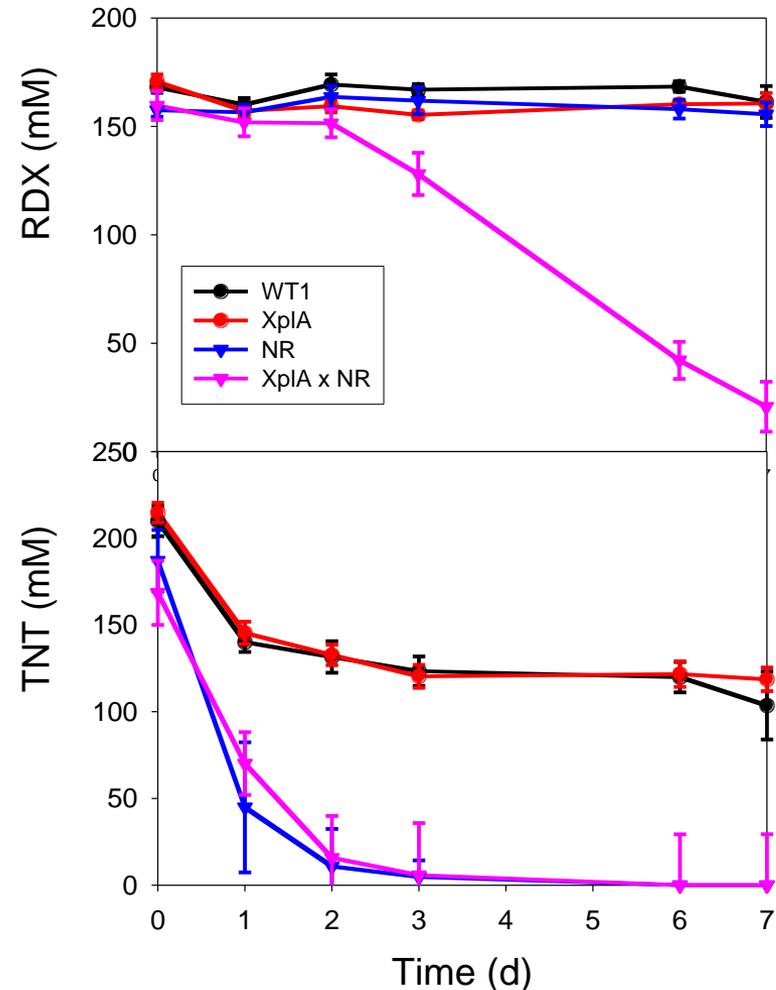
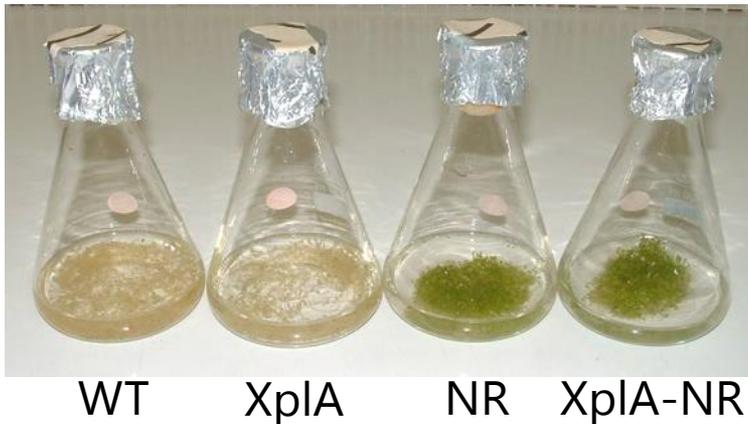


Rylott *et al.* 2006. *Nature Biotechnology* 24: 216

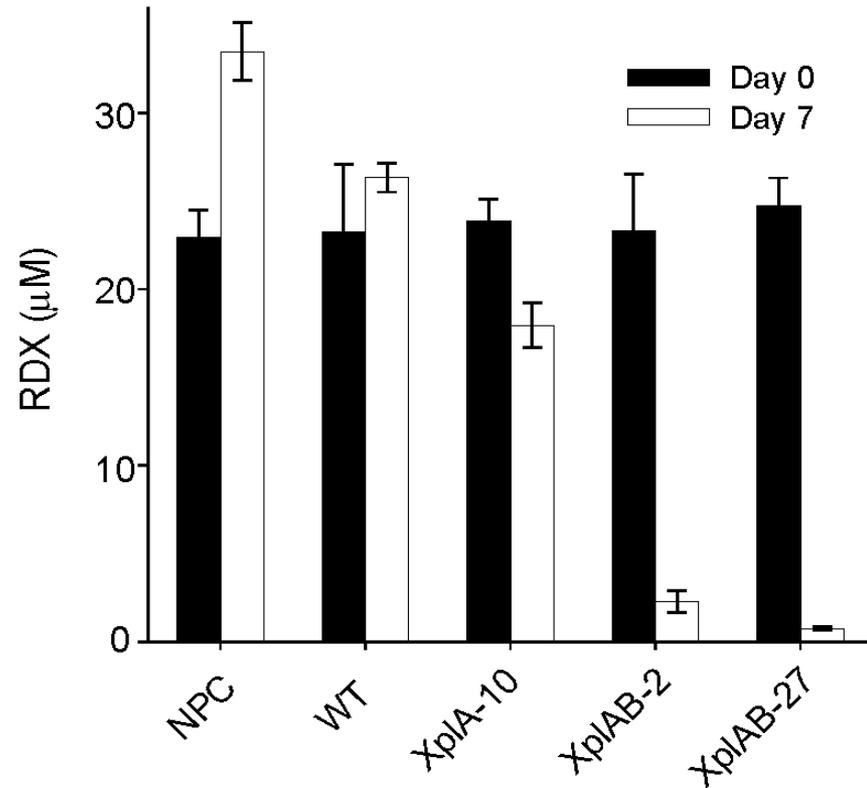
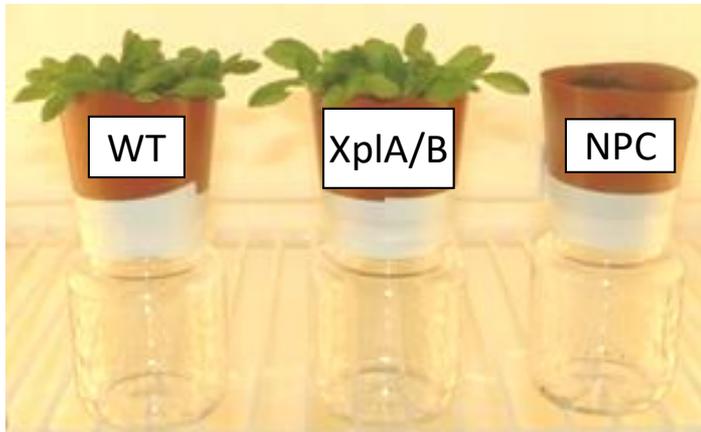
Jackson *et al.* 2007. *Proc. Nat. Acad. Sci. USA.* 104: 16822

# Transgenic *xplA* and *NR* Arabidopsis

*Uptake of RDX and TNT by arabidopsis plants expressing XplA and NR*



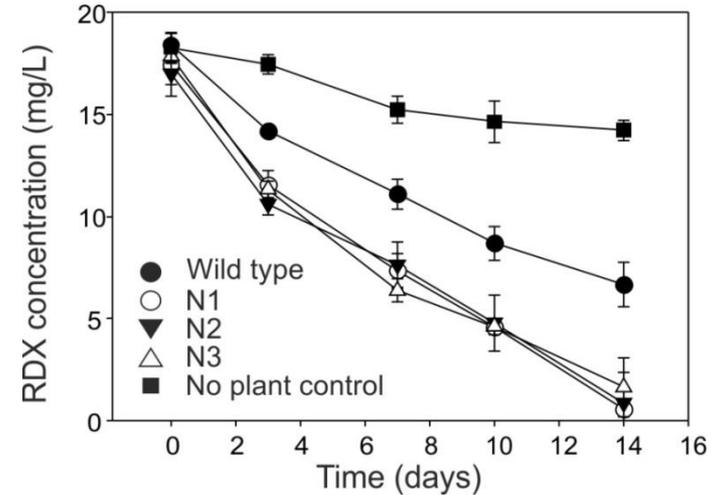
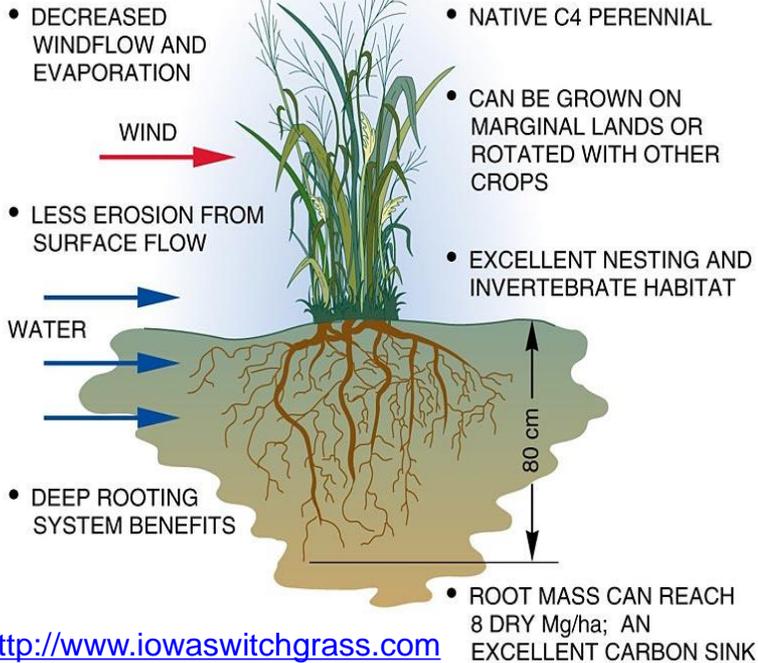
# Soil Leachate Experiment with Transgenic *xplA/B* Plants



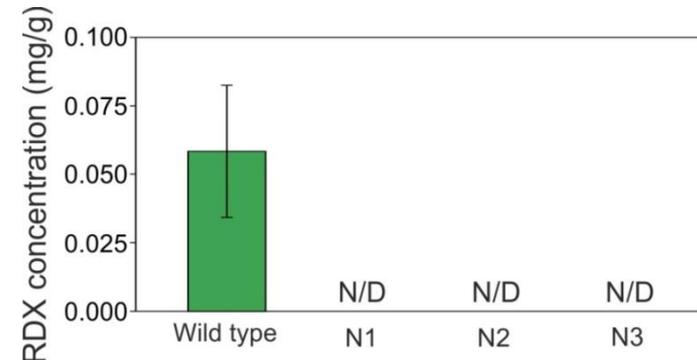
# Transgenic *xplA* Switchgrass

ORNL-DWG 93M-8892

## SWITCHGRASS



RDX by *xplA-xplB-nfsI* transformed switchgrass grown in liquid culture



Concentration of RDX in switchgrass tissue after 14 days

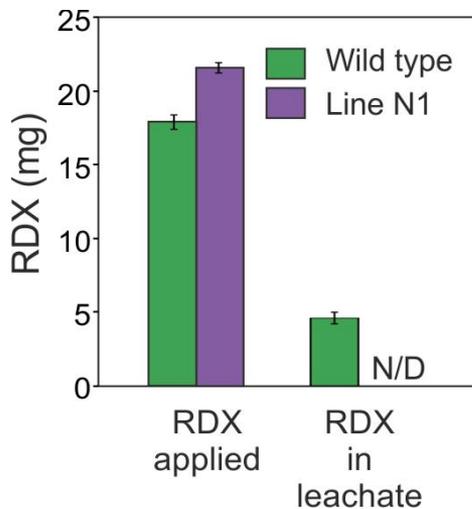
Zhang *et al.* 2017. *Plant Biotechnol. J.*  
DOI: 10.1111/pbi.12661

# Switchgrass Microcosms

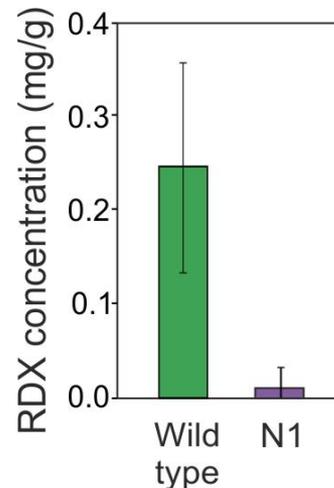
- Testing showed no significant sorption of RDX to microcosm materials
- Substrate contained 3:1 washed gravel and sand



RDX levels in leachate

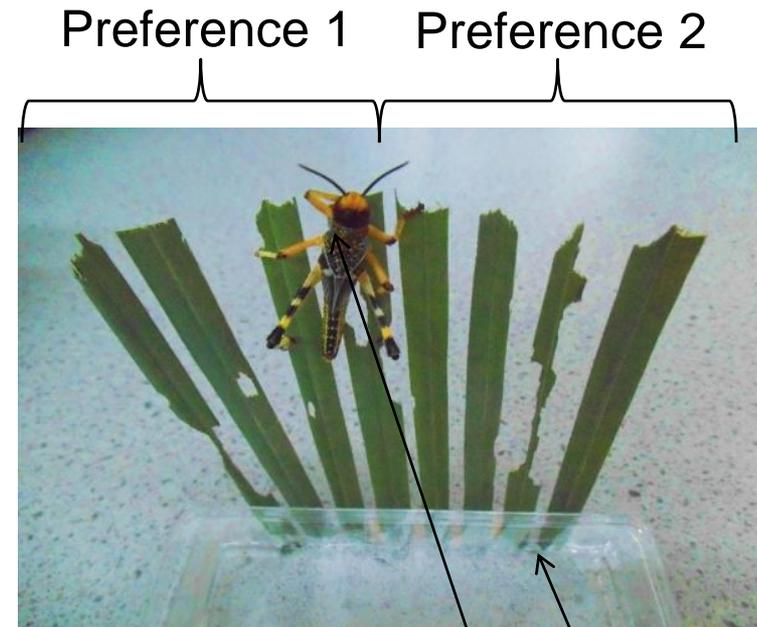


RDX levels in leaf tissues



Zhang *et al.* 2017. *Plant Biotechnol. J.*  
DOI: 10.1111/pbi.12661

# Switchgrass Herbivory Studies

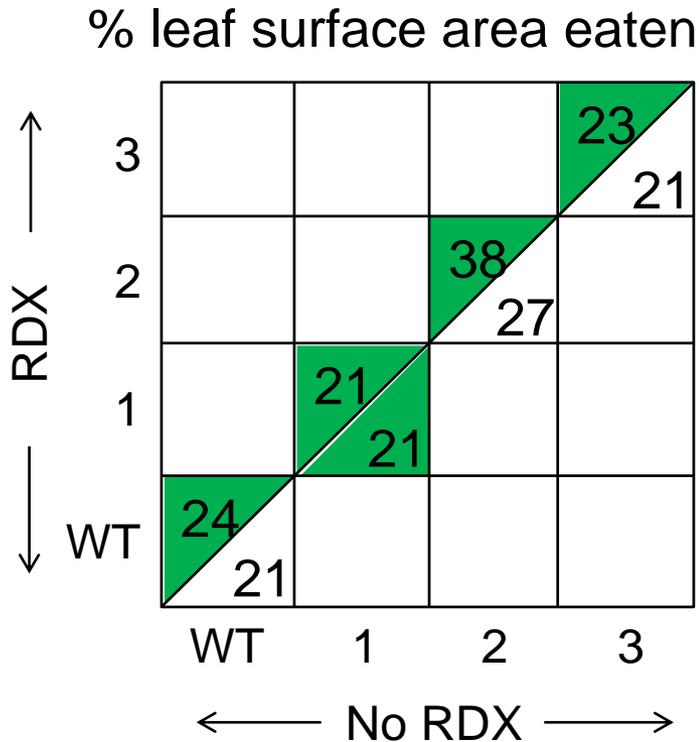


- Arenas set up to compare
  - Preference +/- RDX
  - Preference No RDX: unmodified v modified lines
  - Preference + RDX: unmodified v modified lines
- Locusts left until no more than 50% of one preference foliage eaten
- Surface area of remaining foliage determined

water

5th instar  
locust

# Switchgrass Herbivory Studies: Results

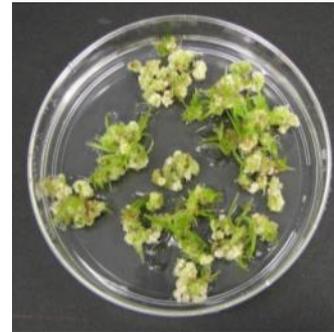


 = preferred vegetation, WT = unmodified, wild type, n = 7

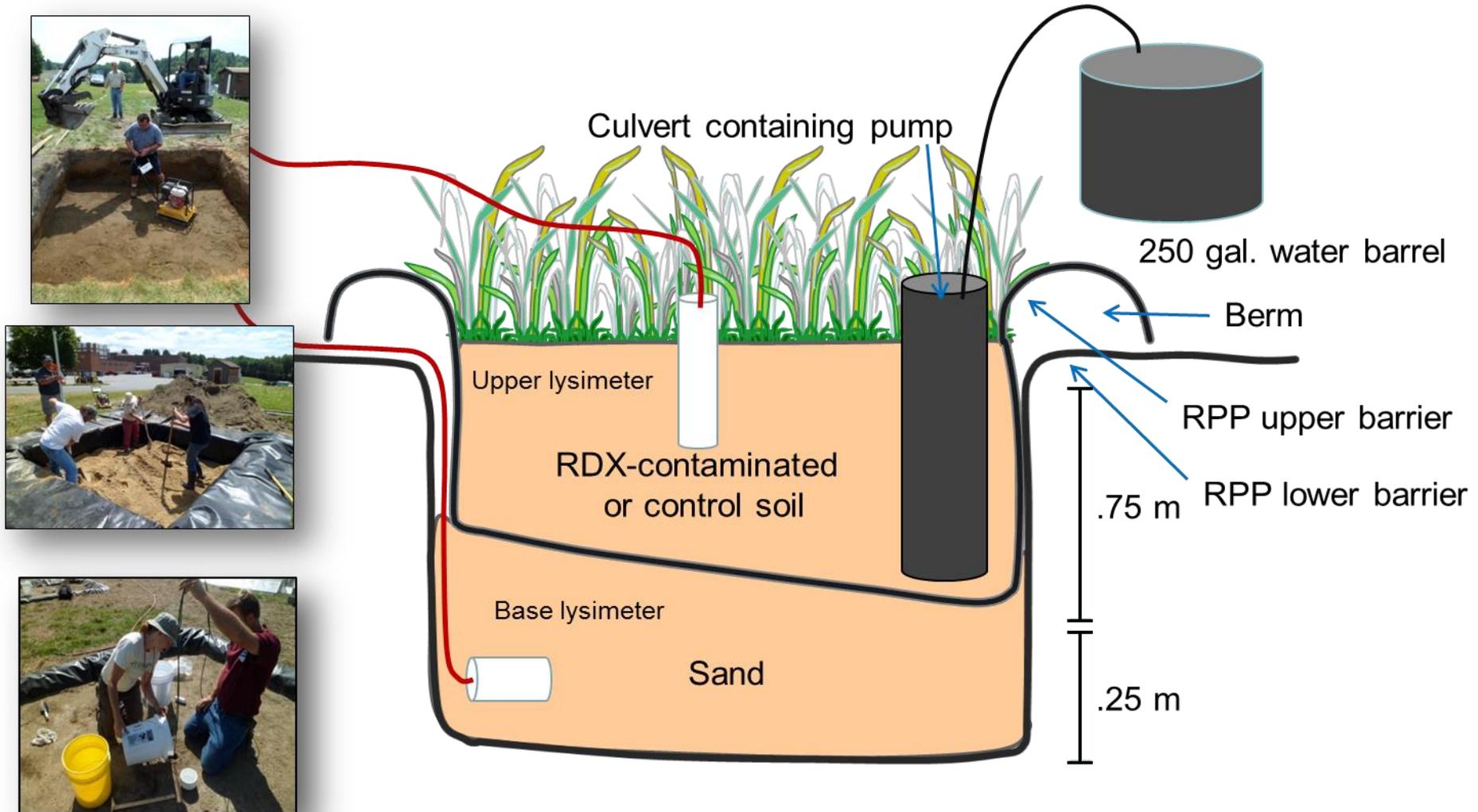
- Presence of RDX has no effect on herbivory preference
- Unmodified leaves preferred over modified lines, regardless of RDX
- Unmodified leaves were softer: a variant from within the seed batch used to produce the modified lines

# Development of Transgenic Grass Lines

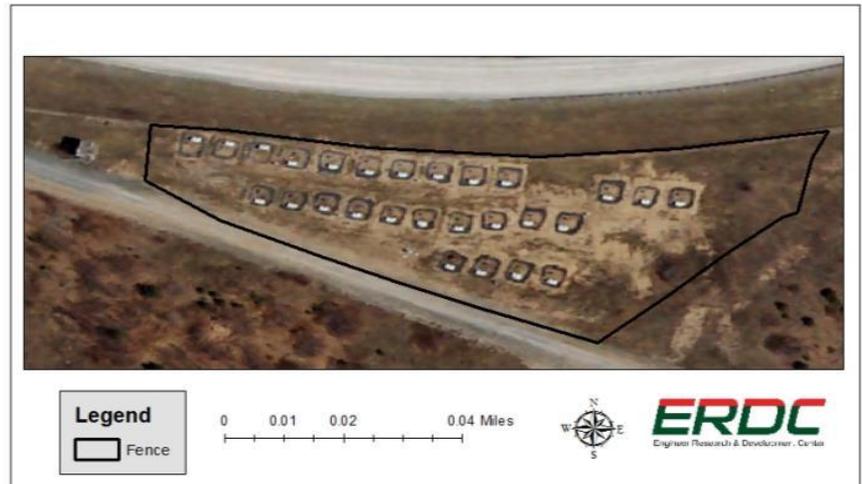
- Mature plants produced
- Switchgrass lines established in glasshouse for trials



# Cross Section of Test Plots



# Field Trials



# Sampling

- Samples of leaf, soil and water have been collected from the plots
- Samples are currently being analyzed for RDX
- Plants were dormant over the winter
- Trials and sample collection are currently continuing



# Summary

- RDX and TNT are major pollutants on military training ranges and RDX can move off range in groundwater
- We have developed transgenic plants that can remove RDX and TNT from soil preventing leaching to groundwater
- We have successfully demonstrated this technology in greenhouse studies
- USDA-APHIS granted a permit for field trials
- Currently evaluating materials at a U.S. military site

# DoD Benefits

- This technology has the potential to provide a self-sustaining, inexpensive, and environmentally friendly method of range restoration that can be used over large areas of land to prevent groundwater contamination
- This technology will allow the land to remain in use with limited closure to military activities
- Specific areas that can benefit from this technology are wide ranging and include firing points, impact areas, manufacturing sites, and demolition areas

# Acknowledgments

- Liz Rylott, University of York
- Stuart Strand, University of Washington
- Long Zhang, University of Washington
- Timothy Cary, ERDC-CRREL
- Tony Palazzo, ERDC-CRREL

# *SERDP & ESTCP Webinar Series*

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<https://www.serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminants-on-Ranges/Protecting-Groundwater-Resources/ER-201436>

<https://www.serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminants-on-Ranges/Protecting-Groundwater-Resources/posted-9-16/ER-1498>

## **Speaker Contact Information**

neil.bruce@york.ac.uk; +44 1904 328777



## Q&A Session 2



The next webinar is on  
August 17, 2017

*Zinc Nickel Dip and Brush Plating*



## Survey Reminder

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