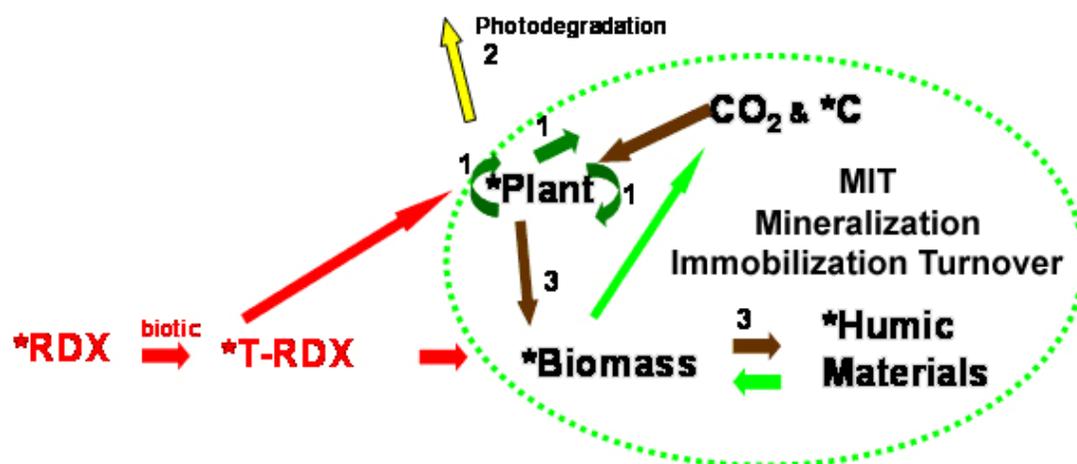




**Fate of Plant Tissue Associated RDX in Surface Soil:  
SERDP ER-1412 Annual Report for 2005**

Charles M. Reynolds, Lee Newman, and John Ferry

March 2006



# **Fate of Plant Tissue Associated RDX in Surface Soil:**

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### **Prepared for:**

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## Project Background

We are using RDX-laden plant tissue to follow RDX humification and fate in soils held at moisture and temperature conditions representative of surface soils on training ranges. We are also using variegated plants to characterize phyto- and photo-degradation in pigmented and non-pigmented tissues.

Results from this research will have wide-ranging implications for military impact ranges and ultimately the cost of treating US sites. This work is clearly aligned with the Joint Interagency Program on Phytoremediation Research described under DOE OBER notification 03-04, and addresses fundamental mechanisms of interactions between plants, physical properties of the contaminant of interest, and soil components to better understand the mechanisms involved in RDX removal from the environment.

RDX has been shown to attenuate readily in sufficiently reduced soils and to a much lesser extent under strict aerobic conditions. Although RDX biodegradation proceeds at reasonable rates under anaerobic to microaerophilic conditions, surface soils such as those found on military training ranges, are generally not reduced sufficiently to support the biodegradation of RDX. As such, the presence of RDX on these ranges can pose a threat to both human and environmental health.

Earlier research has shown that many explosive transformation products bind covalently to soil humic fractions or organic material in compost, thereby minimizing the associated health risks. Previous phytoremediation studies have shown that RDX can be taken up by plants, but that the compound accumulates in the plant rather than being degraded. Plant uptake and any subsequent phytoremediation and/or photoremediation may partially reduce or retain RDX, but some RDX likely will return to the soil as the plant senesces. Humification of plant associated RDX may have significant utility for continued treatment of tissue-associated RDX on military training ranges.

## Objective

Our overall objective is to improve the understanding of RDX transformation in plant tissues and the subsequent cycling of tissue-associated RDX and RDX daughter products among soil mineral and humic fractions following plant senescence. To accomplish this, we will look at plants within the family *Labiataea* (genera *Mentha* and *Coleus*) to determine their relative sensitivities to RDX and to determine the uptake rates for RDX for the selected plants. We will then use those plants with high uptake rates to follow RDX humification and fate in soils held at moisture and temperature conditions representative of surface soils on training ranges.

Our hypothesis is that environmental risks from RDX at military training ranges can be reduced, and possibly eliminated, through a series of coupled processes involving plant uptake, plant enzyme mediated transformation, photodegradation in the plant, and finally humification of plant-tissue-associated RDX conjugates into soil organic matter after plant senescence and leaf drop. Although the effect of each individual process may be small, the combined effects of the processes taken as a system for sustainability may have a significant impact in RDX residues on surface soils. If so, they may lead to feasible range sustainability management practices.

Mechanistically, pigments and aromatics in plant tissue may control or alter plant-related transformations and photodegradation. Bioavailable carbon from decaying plant tissue may be critical in driving soil humification processes. For these reasons, we have proposed to study the processes as a system. A diagram of the processes is shown in Figure 1.

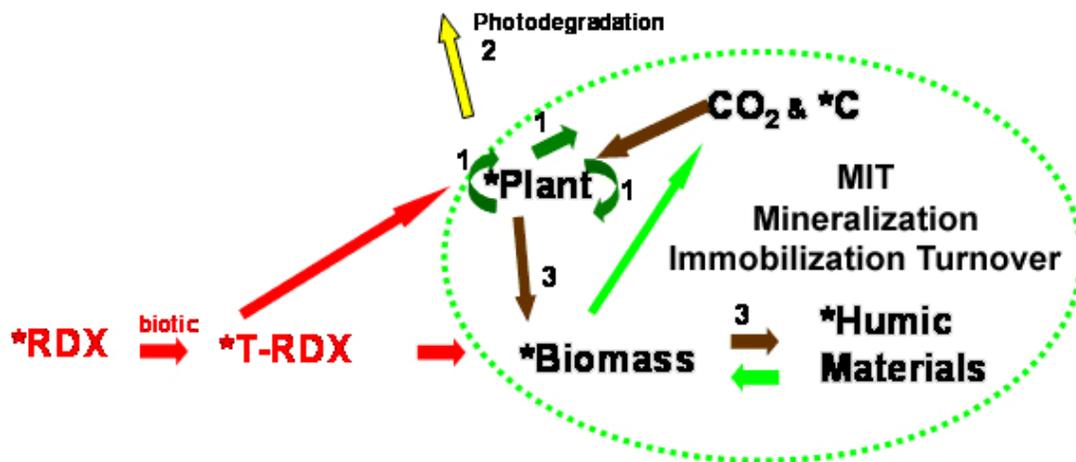


Figure 1. Conceptual fate of RDX carbon in soil-plant-sunlight-microbial system. We are addressing processes 1-plant synthesis and catabolism, 2-photodegradation, and 3- humification of plant-tissue-conjugated RDX carbon in conjunction with Mineralization-Immobilization Turnover (MIT).

Much is known about RDX fate in soils and its uptake by plants. The initial microbially driven degradation steps for RDX in soil at defined conditions, shown in red in Figure 1, have been characterized by others. Although degradation in the plant does not appear to provide a major transformation pathway in plants studied, it does occur. Much RDX is conjugated into larger molecular weight compounds by plants in a process described as a “green liver” function and believed to be part of plant detoxification strategies. The pathways determining RDX fate in plant tissue are less well defined. This project focuses on understanding the occurrence and magnitude of processes denoted by arrows 1, 2, and 3 in Figure 1. To address these gaps, inherent in our hypothesis are the concepts that:

- Process 1. RDX fate in plant tissue is subject to unique plant enzyme systems that may be associated with synthesis of plant pigments or aromatic compounds.
- Process 2. Photodegradation in plant tissue may vary with pigment levels in the tissue, and this phenomena may be a useful tool for understanding occurrence and magnitude of photodegradation of RDX in plant tissue
- Process 3. Humification of plant-tissue-associated RDX conjugates is a component of and is influenced by natural carbon cycles. Carbon cycles in soil include mineralization, or loss of carbon from the system as CO<sub>2</sub>, and immobilization, or fixing carbon into the soil organic fraction. These processes occur simultaneously, a net process termed Mineralization-Immobilization Turnover (MIT), and reach a dynamic equilibrium. The equilibrium can be shifted towards mineralization by processes such as tilling, or towards immobilization by processes such as carbon

additions. On ranges, we may be able to alter humification processes by adding high levels of carbon, such as from plant senescence.

To evaluate this hypothesis, we have three main objectives or tasks:

**(1) Compare uptake and removal rates by herbaceous plants such as mint and coleus.** Mint and related plants (family *Labiataea*), with their unique metabolic pathways, will be exposed to RDX, and the plants examined to determine if these pathways can be used to facilitate the breakdown of RDX. Task 1 will take place during the first 18 months of the project duration, with additional plants grown as needed to accomplish tasks 2 and 3. We will determine the RDX uptake rates for the plants studied and select plants with limited toxicity and high uptake capacity for RDX.

**(2) Determine the fate of RDX in selected plants.** We will compare RDX accumulation and degradation rates between the two genera of plants, compare degradation in pigmented (green) tissue vs. non-pigmented tissues, and compare degradation in green (chlorophyll) vs. red/purple (anthocyanins) portions of the plants. This work will be done primarily during the last two years of the project, with the ultimate objective of selecting the best plants to use for RDX degradation.

**(3) Track and identify fate of <sup>14</sup>C-RDX added either directly to soil or from plant tissue.** In the first two years of the project we will characterize the relationships among RDX transformation, soil water potential, and bioavailable carbon at temperatures representative of surface field soils. We will determine if humification of RDX-derived carbon occurs and is related to mineralization-immobilization turnover of natural soil carbon, a readily measured process that can be influenced with large-scale practices. In the final year, we will investigate the fate of plant-associated RDX as it decomposes in both reducing and humifying conditions representative of those in surface soils.

## Technical Approach

This project brings together three areas of RDX removal and degradation—plant uptake, photodegradation, and humification—and builds on preliminary work done by members of this research team and many others in this field. We are working to better understand the biochemical and physiochemical transformations of RDX within the plant matrix, as well as how the parent compound and metabolites interact with components of the soil matrix.

This study combines three approaches for the removal and degradation of RDX in contaminated soils: (1) plant uptake and removal, (2) accumulation, phyto- and photo-degradation rates, and (3) humification.

**(1) Plant Uptake and Removal.** Mint and related plants (family *Labiataea*), with their unique metabolic pathways, will be exposed to RDX, and the plants examined to determine if these pathways can be used to facilitate the breakdown of RDX. There is also the option of selecting plants, both from the family *Labiataea* and other plants such as *Coleus*, that have relatively large un-pigmented areas.

**(2) Accumulation, phyto- and photo-degradation rates.** Pigmented and non-pigmented mint tissues, as well as tissues from variegated *Coleus* plants, will be examined to determine if the

movement of the RDX to a leaf tissue that exposes the RDX to light at much higher levels that would be present in the soil or in pigmented tissue can lead to photodegradation of RDX. Rather than looking at the transmission spectra of the leaves, we will be exposing the plants to various wavelengths of light. It is well documented that RDX degrades when exposed to UV and shorter wavelengths of light; it is more stable when exposed to the shorter, red wavelengths. Since plants can grow and thrive under red light, we will expose the green/white coleus plants to RDX while growing under red light. We will then compare the RDX accumulation patterns to those seen under white light to determine if the differences seen are due to photodegradation or some combination of phyto-photodegradation pathways.

(3) Humification. Tissue from plants with high uptake rates will be incorporated into ongoing humification studies that are investigating the fate of plant-tissue-associated RDX as plant tissue falls to soil. This three-tiered approach will cultivate the development of natural remediation systems for the remediation of RDX that are sufficiently robust for widespread use on impact ranges and based on well-defined processes.

The work for approaches (1) and (2) is being conducted primarily by Dr. Lee Newman and Dr. John Ferry at the University of South Carolina. The comparisons of uptake and removal rates (1) are being done in the laboratory and greenhouses that Dr. Newman has available. Initial plant assessments are done in the laboratory to have better control of the conditions. Large-scale production of plant material for analysis is conducted in the greenhouse, which will afford more space. Plants that have at least a moderate level of resistance to RDX toxicity, as well as the highest uptake rates are being used for the remaining tasks. The comparison of RDX accumulation and degradation rates (2) are a joint project between Drs. Newman and Ferry, with the bulk of the analysis being done in the Chemistry Department at USC. The humification work (3) is being conducted by Dr. Mike Reynolds at CRREL using tissue from the plants grown in soil amended with  $^{14}\text{C}$ -labeled RDX. Soil humification studies are done in the laboratory under controlled conditions.

Using  $^{14}\text{C}$  tracers to follow the fate of RDX in plant tissue and soils is critical. Cycling of  $^{14}\text{C}$  in the soil will largely be a function of MIT. If MIT rates are slow,  $^{14}\text{C}$  flux among different soil compartments may also be slow.

## Project Accomplishments

### **Task 1. Compare uptake and removal rates by herbaceous plants such as mint and coleus**

We have completed the first two of the three sub-tasks under this first objective. The third sub-task is still in progress.

- 1.1 Investigate plants within the family Labiatea (genera *Mentha* and *Coleus*) to determine their relative sensitivities to RDX
- 1.2 Determine the uptake rates for RDX for the selected plants
- 1.3 Select plants with limited toxicity and high uptake capacity for RDX

For plants, we have shown a difference among plants in RDX uptake and RDX toxicity. The coleus plants do not seem to have problems growing hydroponically in high concentration RDX solutions, but some of the scented mints show definite signs of toxicity. We are currently correlating this observation with the water uptake rates of the plants, and once extractions are completed, will determine if the plants are more sensitive or if they are showing toxicity due to higher uptake rates. Interestingly, spearmint plants, which are so far the most sensitive to RDX toxicity, show a 29% higher water uptake rate per unit plant tissue in the exposed plants than the control plants. This analysis is still being done for the other exposed plants.

The correlation between water uptake and RDX uptake is problematic in soils as there are many competing factors for uptake. We have begun our studies by looking at plants in hydroponic solutions so that we can circumvent this problem. Concentrations of RDX and volumes of water will be determined at the start and end of exposure periods. This enables us to determine the water/RDX uptake ratios that the plants are capable of performing.

We are currently working with the Department of Energy's Savannah River Site and other government sites to obtain soils contaminated with RDX to initiate soil based uptake experiments with various plants. Large plants have been overwintered in the greenhouse, so as soon as we obtain the soil we can start this experiment without having to wait for plants to be available commercially.

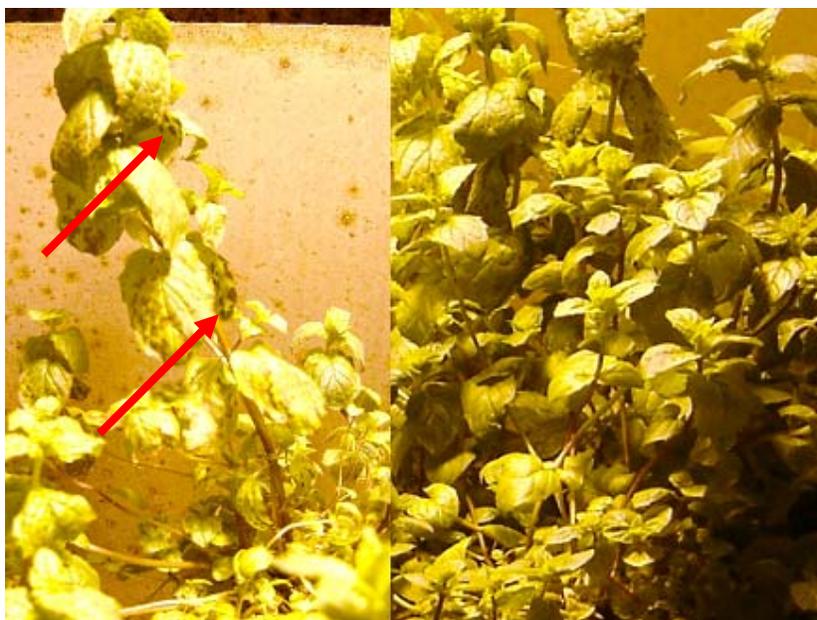


Figure 2. Image of RDX-exposed spearmint plants vs. control plants prior to takedown. Note brown lesions on exposed plants.

### **Task 2. Determine the fate of RDX in selected plants**

We have begun work on the first three sub-tasks in this area:

- 2.1 Determine the differences in RDX phyto-degradation in the green tissues of plants of the family Labiatea, specifically those plants in the genera *Mentha* (mint) and *Coleus* (coleus)
- 2.2 Determine the difference in RDX photodegradation and phytodegradation in pigmented (green) vs. non-pigmented plant tissues
- 2.3 Determine the difference in RDX phytodegradation in *Coleus* plants in plant tissues pigmented by chlorophyll (green) or anthocyanins (red/purple)
- 2.4 Select the best plants to use for RDX degradation.

### **Uptake and Accumulation**

We have shown differences in leaf accumulation between three plants studied, coleus, peppermint, and tobacco (Table 1). The coleus appeared to have the lowest accumulation of RDX in the leaf tissue, and this appears to be because RDX concentration within the white tissue is lower than in the green, pigmented tissues.

Table 1. RDX uptake and accumulation in three plants.

	water use (ml)	Total RDX taken up (mg)	RDX in leaves (mg)	% RDX taken up in leaves
tobacco	275	1.54	0.95	61.95
	250	1.40	1.44	102.86
peppermint	635	3.56	2.83	79.48
	850	4.76	3.53	74.20
	700	3.92	2.17	55.26
coleus green tissue	245	1.37	0.27	19.6
	315	1.76	0.59	33.7
	135	0.76	0.16	21.4
coleus white tissue	245	1.37	0.06	4.4
	315	1.76	0.38	21.5
	135	0.76	0.03	4.2

We have shown preliminary differences in RDX concentrations dependent on the color of the leaf tissue when looking at coleus plants with tricolored leaves (Table 2). We are repeating this experiment for verification as the original sample size was relatively small.

Table 2. Differences in RDX update in tricolored leaves of coleus.

	RDX conc. (ppm)	Standard deviation
Green tissue	1.22	0.52
White tissue	0.75	0.08
Red tissue	0.29	0.09

To investigate the possibility of preferential accumulation of RDX in different areas of the leaf tissue, we have grown plants that have different pigmentation patterns as well as solid green coleus, and have exposed them to RDX. The pigmented leaves were sectioned so we can extract the different pigmented areas separately. The solid green coleus was also exposed to RDX, and the leaves, although solid green, were sectioned in the same manner as the differentially pigmented leaves. In this way, we will be able to determine if the differences in RDX accumulation are due to depositional differences or the pigmentation. We are in the process of extracting the plant tissues and will be analyzing them as soon as the extractions are done. We have also exposed plants where the entire leaf is pigmented with anthocyanins, and will be extracting those plants to determine percent recovery of RDX and percent accumulation within leaf tissues. We have also looked into extracting the anthocyanin pigments to add to the reaction mixes to determine their potential role in photodegradation of the RDX

### Photodegradation Studies

We have performed a multivariate photolysis of RDX in the presence of glutathione, glutamine, cysteine, sucrose, and ascorbate. The effort involved observing RDX photodegradation over a 12

hr period, sampling at 3 hr intervals, incorporating a total of approximately 320 photolyses measurements. In every sample, RDX photodegraded; however, it did not correlate to the concentration of these molecules (Figure 3). From this, we have concluded that RDX will photodegrade given exposure to 300 nm-400nm photons but that degradation is a wholly intramolecular process and is probably not related to the molecular content of the cytosol.

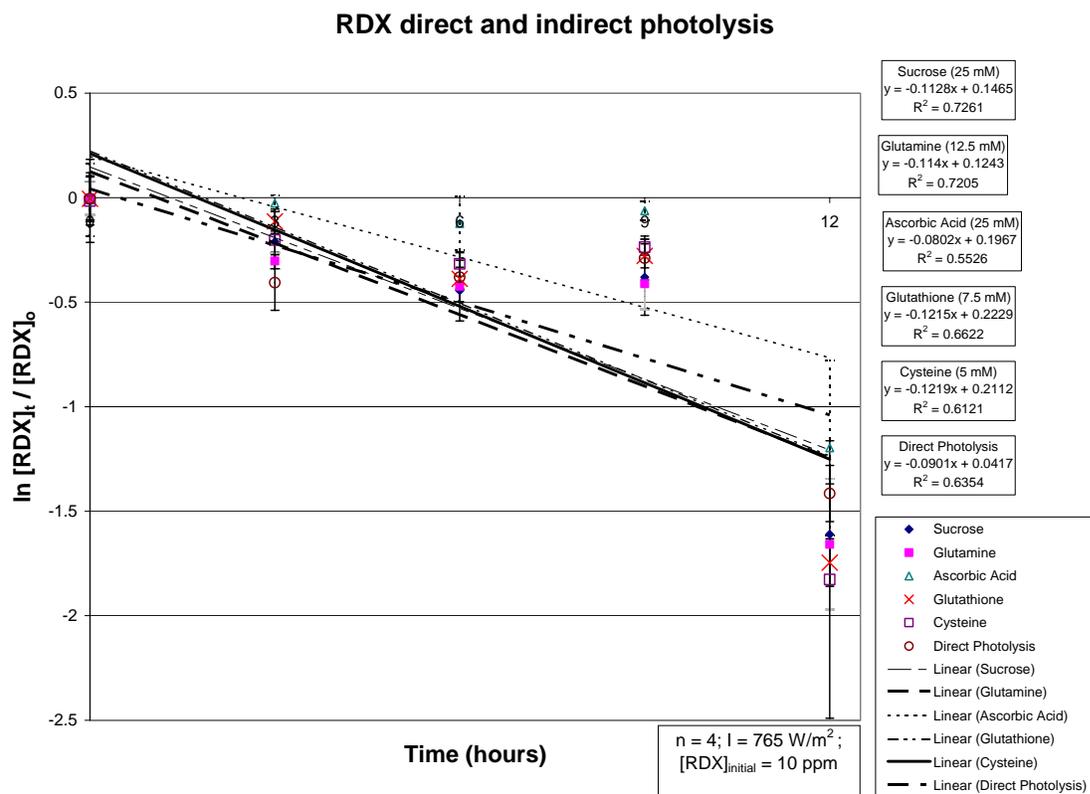


Figure 3. RDX photolysis does not correlate with the presence of several different cytosol components.

We ran corresponding matrices for a series of nitroaromatics (initially nitrobenzene; 3-nitroanisole; 4-nitroanisole; 3-nitrotoluene; 4-nitrotoluene; 3-nitrophenol; 4-nitrophenol; 1-chloro-3-nitrobenzene, and 1-chloro-4-nitrobenzene). Nitrobenzene photolysis appears to be quite sensitive to cytosol components, with glutathione taking the lead in promoting photodegradation (Figure 4). Screening the activity of other nitroaromatics is ongoing. This strategy of screening cytosol components for their impact on intermolecular photodegradation helps us identify a photoremediative approach where the plant selection can be optimized to promote abiotic photodegradation in the plant. None of these components have an effect on RDX or NB in the “dark” over a period of weeks. These experiments were not optimized to probe humification processes.

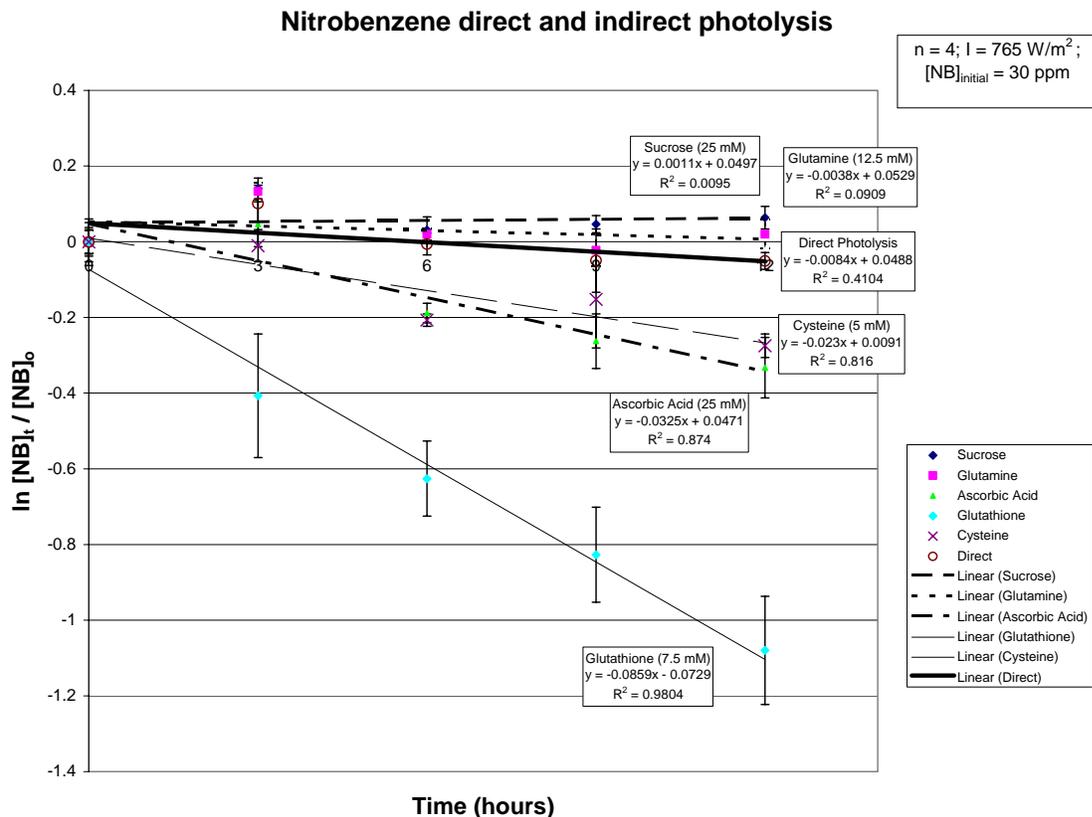


Figure 4. The photodegradation of nitrobenzene correlates strongly with the presence of glutathione. Ascorbate and cysteine may also be significant.

We are harvesting more complex structures (membranes, organelles, etc.) for use in photodegradation matrices. We hope that will happen this next quarter (Jan 2006-April 2006). We are growing bulk amounts of tobacco to use as the source of chloroplasts to ensure active organelles.

We performed the corresponding dark controls for RDX stability in contact with solutions of the same. In the absence of UV radiation, RDX was stable for the duration of the experiment (2 weeks). The implication is that RDX degradation in the plant is a function of incident solar radiation and that these commonly occurring reductants do not react with it at all in the absence of enzymes. There are implications for humification in this, but we are not sure what they are.

We are continuing to work on developing a rapid screening process for secondary photoactivity in the presence of important cytosol components based on fluorescence quenching of nitroaromatics. The screen is based on plate reader technology and will allow us to assay 100 or more cytosol components at a time, or alternatively tens of different micropollutants with a more limited cytosol model. The photoexcited state of nitroaromatics is often strongly oxidizing, and we will be adding a variety of cytosol components to nitroaromatic solutions to see if they quench the native fluorescence. If they do, then it is possible that the quenching is reactive. Although this assay will deliver false positives for measuring the reactivity of cytosol

components, it won't deliver false negatives, and will help strategize plant selection to maximize potential secondary photodegradation. This is a new approach for us, and the opportunity has come because of a recently funded NSF grant that allowed the purchase of the reader.

### ***Task 3. Track and identify fate of <sup>14</sup>C-RDX added either directly to soil or from plant tissue***

The underlying hypotheses that task 3 addresses are:

RDX in plant tissue, which is predominantly in conjugated forms due to plant-based processes, can be further irreversibly tied to soil organic matter following plant leaf senescence.

Incorporation by humification of the RDX residues into soil organic matter will be related positively to the general turnover of soil organic fractions, a process termed mineralization-immobilization turnover (MIT).

Consequently, soils with high MIT will be better at humifying RDX residues than are soils with low MIT.

To test these hypotheses, we have completed the three sub-tasks in this area and are addressing an additional sub-task to better understand the toxicity of humic material leachate following RDX incorporation, relative to the same without RDX incorporation. This was an action item in the spring 2005 In Progress Review.

- 3.1 Characterize the relationships among RDX transformation, soil water potential, and bioavailable carbon at temperatures representative of surface field soils.
- 3.2 Determine if humification of RDX-derived carbon occurs and is related to mineralization-immobilization turnover of natural soil carbon, a readily measured process that can be influenced with large-scale practices.
- 3.3 Investigate the fate of plant-associated RDX as it decomposes in both reducing and humifying conditions representative of those in surface soils.

### **Methods**

A schematic of the approach we used is shown in Figure 5 below. In brief, two soils, with different organic matter contents, were incubated with <sup>14</sup>C-labelled RDX applied directly to the soil, and, in matched treatments, with <sup>14</sup>C-labelled RDX that first was incorporated into plant tissue by growing plants in <sup>14</sup>C-labelled RDX in soil and then adding the plant tissue to the soil. The soils were incubated at constant temperature and moisture conditions for periods of approximately five months. Evolved CO<sub>2</sub> and <sup>14</sup>C-labelled CO<sub>2</sub> were monitored by pre and post scrubbing the headspace air for CO<sub>2</sub>. Periodically, soil cores were taken and analyzed for RDX. Analysis fractions included acetonitrile-extractable RDX in the soil, <sup>14</sup>C in the microbial biomass fractions, and <sup>14</sup>C in the soil organic matter, which was fractionated further into fulvic, humic, bound humic and bound lipid fractions.

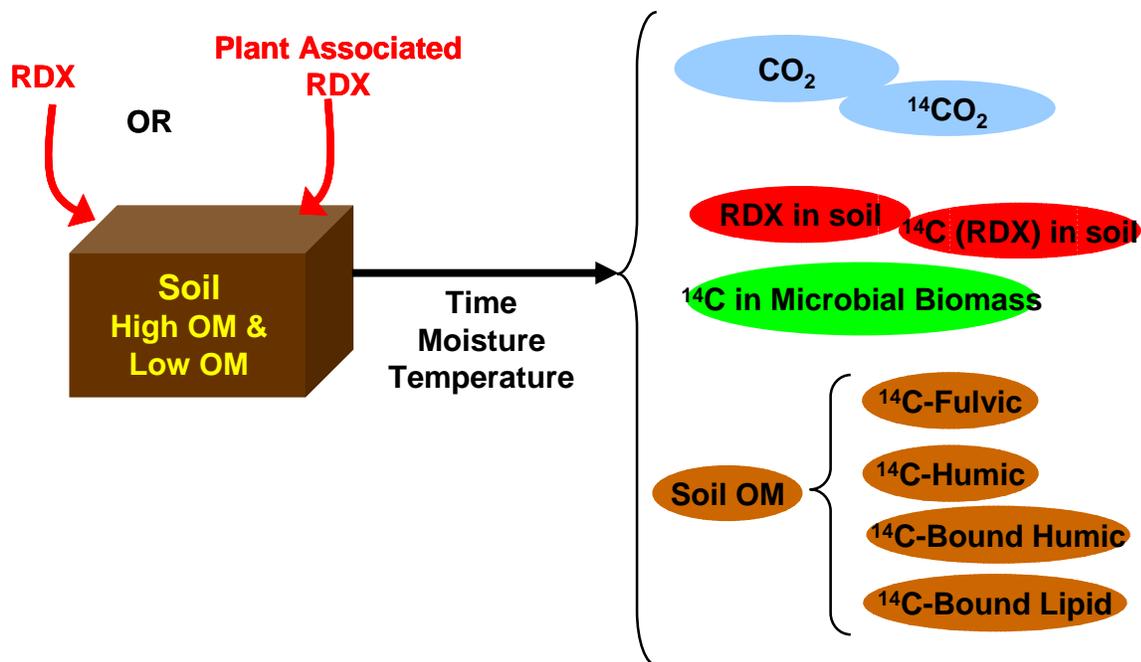


Figure 5. Schematic of approach to track and identify fate of  $^{14}\text{C}$ -RDX added either directly to soil or from plant tissue

## Results

The summarized results are shown below in Figures 6 and 7.

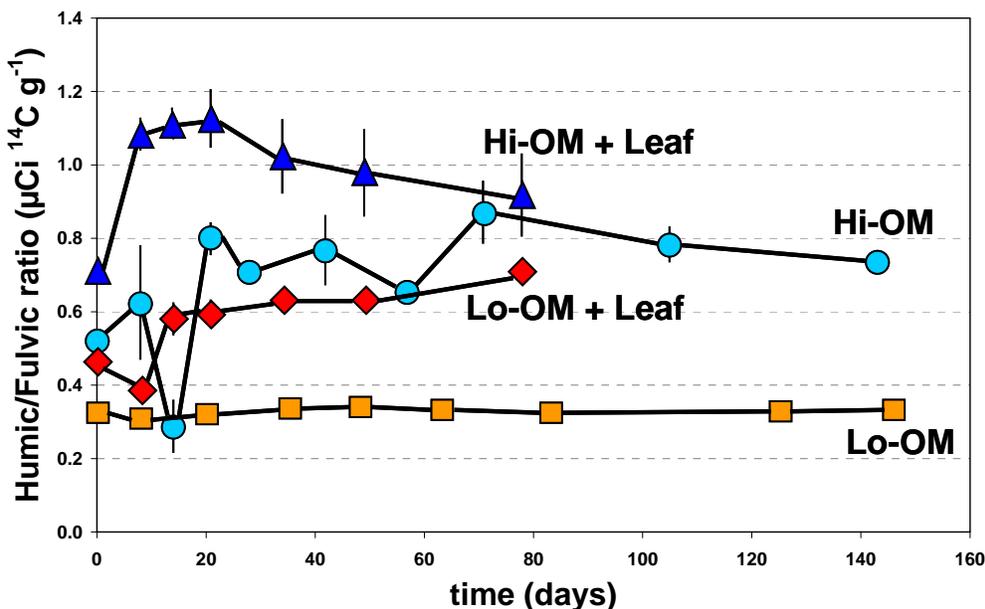


Figure 6. Humic/fulvic ratios in high and low OM soils treated with either  $^{14}\text{C}$ -labelled RDX applied directly to the soil or  $^{14}\text{C}$ -labelled RDX that was incorporated into plant tissue by growing plants in  $^{14}\text{C}$ -labelled RDX in soil (+ Leaf).

When  $^{14}\text{C}$ -RDX was applied directly to the soil, the amount of  $^{14}\text{C}$  in the organic material, expressed as humic:fulvic ratio, was significantly higher in the high organic soil than in the low organic soil. However, when  $^{14}\text{C}$ -RDX was applied as plant-tissue associated RDX (+Leaf in Figure 6), the amount of  $^{14}\text{C}$  label that was bound into the humic:fulvic ratio reached similar ratios for both soils by 80 days of incubation. These data support the hypothesis that MIT is driven by bioavailable carbon, and MIT in turn drives humification, or soil organic matter association of  $^{14}\text{C}$  derived from RDX.

Further supporting data are shown below in Figure 7. These data show that microbial biomass is relatively constant and uniform for both the control (no RDX, no plant tissue) and the RDX additions in the high organic matter soil during the incubations, suggesting that the system is in near steady state condition microbially. However, in the low organic matter soil, both the control and the RDX treated soils have highly variable microbial biomass, suggesting greater changes in the microbial populations.

The lower graphs in Figure 7 show the cumulative  $\text{CO}_2$  flux from the soils. Without plant tissue additions, the high organic matter soil has greater evolved  $\text{CO}_2$  than does the low organic soil. Addition of plant tissue increases evolved  $\text{CO}_2$  for both soils, and the  $\text{CO}_2$  evolution curves are nearly identical for both soils. These data show that the addition of readily bioavailable carbon, such as plant tissue, results in similar evolved  $\text{CO}_2$  for both soils and overcomes inherent soil organic matter differences. These data are also in agreement with, and support, greater MIT and greater  $^{14}\text{C}$  incorporation in the humic:fulvic fraction of the soils.

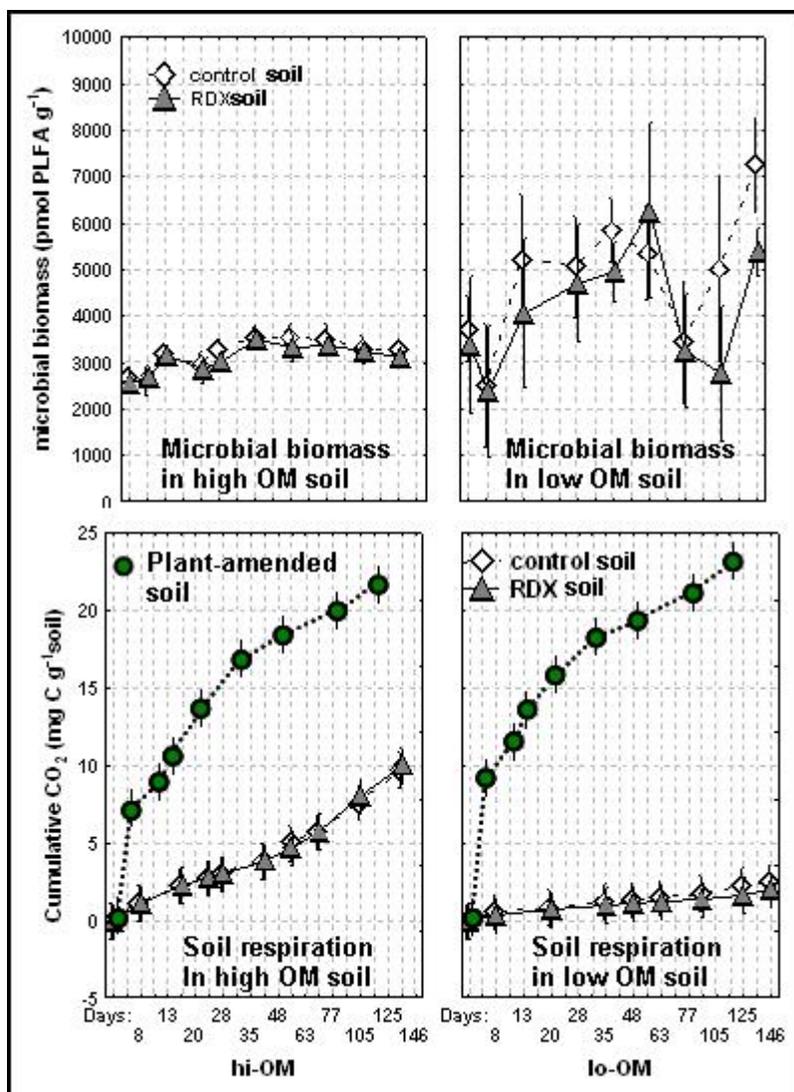


Figure 7. Upper two graphs: Microbial biomass in high organic soil and low organic soil, with and without RDX additions. Lower two graphs: Soil respiration expressed as evolved CO<sub>2</sub>, in high organic soil and low organic soil, with and without RDX additions, and with plant tissue and associated RDX amendment.

The practical implications of these data are that humification of RDX into the soil organic fraction is enhanced by: 1.) “preconditioning” the RDX by plant-associated conjugation processes and 2.) increasing MIT in the soil. Both of these enhancements can be favorably influenced by exploiting the plant-uptake, conjugation, and plant senescence cycles.

### Ongoing Efforts

In response to the feedback from the spring 2005 review, we have designed a test for the toxicity of leachate from humic material with and without RDX incorporation or humification. There are limits imposed by the nature of the previous studies in which we generated soil that had RDX and plant-associated RDX bound to or in the humic fraction. Because the mass of soil remaining from the earlier humification tests is limited, we sought a modified toxicity test that could be

done with minimal soil and yet had relatively high sensitivity. Additionally, earlier work on the toxicity of leachate from other explosive treatment studies, notably composting, have shown that control, or non-explosive amended compost had relatively high toxicity that appeared to be, for the tests used, inherent to the compost.

Our current approach is an algal-based test that requires small masses of soil, is relatively sensitive, and is based on *Selenastrum capricornutum* growth inhibition. We have arranged for these toxicity screenings to be done at the Environmental Laboratory in Vicksburg, Mississippi, by Dr. Laura Inoye.

Due to the small amounts of soil that remain from the earlier humification incubations, there are constraints on the comparisons that we can make. The comparisons are: soil vs. plant; low OM vs. high OM, pre-incubation vs. post incubation, and RDX vs. no RDX (Figure 8).

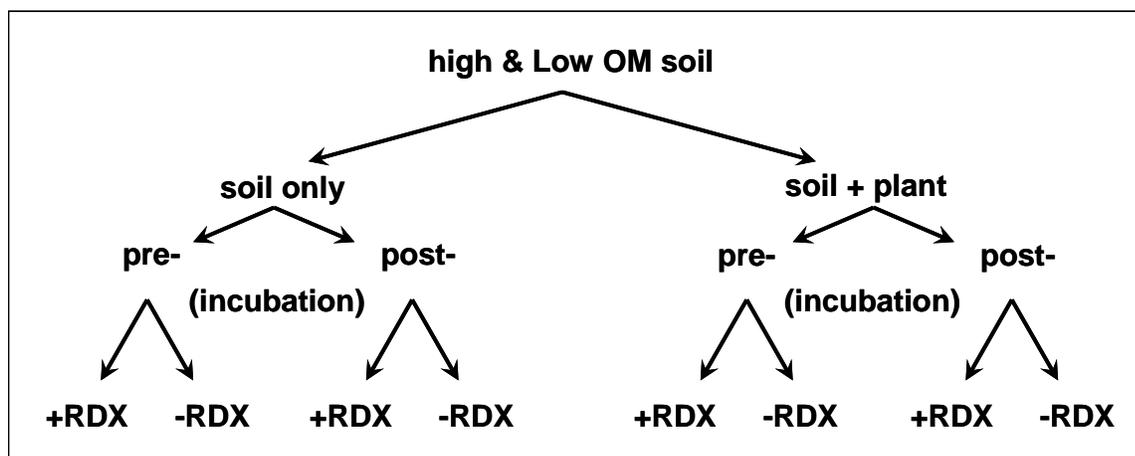


Figure 8. Treatments that are being tested using a modified leachate toxicity test based on the growth inhibition of the algae *Selenastrum capricornutum*.

A synopsis of the methods follows. Available soils will be processed via a modified TCLP batch assay. A slurry of 5g soil in 40 ml H<sub>2</sub>O is shaken at 30 rpm for 24 hours. The slurry is then centrifuged at 10,000 x g for 10 minutes to pellet the soil. The supernatant is recovered and preserved at 4°C for the micro-algae toxicity test. An additional 40 ml of H<sub>2</sub>O is added to the soil pellet, and the supernatant recovered and stored. The soil pellet is then extracted with 40 ml of 2N acetic acid and the supernatant collected and stored. To date we have acquired leachates from initial and final high and low organic matter soils with and without the RDX supplement.

We also will investigate fractionating the leachates via dialysis and/or size exclusion chromatography to identify the molecular weight fraction or fractions that contain the <sup>14</sup>C-RDX carbon.

## Summary

To date, for soils incubated under non-saturated, aerobic conditions representative of surface range soils, we have observed differences in <sup>14</sup>C -RDX fate in high organic matter (OM) soils relative to low OM soil. Greater OM is related positively to greater <sup>14</sup>C in the bound-humic fraction.

Additionally, our data show that adding the  $^{14}\text{C}$  conjugated in plant material to the high OM soil is, after 80 days, yielding  $^{14}\text{C}$  in humic-fulvic ratios that are similar to ratios measured after adding  $^{14}\text{C}$  -RDX directly to the soil.

For the low OM soil,  $^{14}\text{C}$  in the humic-fulvic ratio after adding plant-associated  $^{14}\text{C}$ -RDX more closely follows the high OM soil, and similar amounts of  $^{14}\text{C}$  (from RDX) becomes associated with bound humic fractions.

These data support the concept of humification and suggest that MIT processes, which can be favorably influenced by soil OM management, may have utility as a tool for affecting the fate of RDX residues in favorable ways. Current studies are investigating changes in toxicity of leachate from soil humic material that has RDX moieties incorporated, relative to non-RDX humic material.

## **Appendix**

### ***Peer-reviewed Journals***

Reynolds, C.M., D.B. Ringelberg, and S.L. Larson. (in preparation). Fate of plant-tissue associated RDX in aerobic soils. (based on above data in Task 3).

### ***Technical Reports***

Ringelberg, David B., Charles M. Reynolds, Lawrence B. Perry, and Karen L. Foley . 2005. Effect of acetonitrile on RDX biodegradation in an unsaturated surface soil. ERDC/CRREL TR-05-5.

Ringelberg, David B., Charles M. Reynolds, Karen L. Foley, and Lawrence B. Perry. 2005. Microbial community shifts associated with RDX loss in a saturated and well-drained surface soil. ERDC/CRREL TR-05-4.

### ***Published Technical Abstracts***

Reynolds, C.M., D.B. Ringelberg, L. Newman and S.L. Larson 2005. Humification of Plant-Tissue Associated RDX. In Program Guide, Partners in Environmental Technology Technical Symposium and Workshop, Washington DC, 29 November – 1 December 2005, SERDP-ESTCP, Arlington, VA. p F-27.

Reynolds, C.M., L.A. Newman, D.B. Ringelberg and S.L. Larson.2005. Invited Presentation – “Plant-Soil-Microbial Cycles Leading to Live-Fire Range Sustainability”, Phytoremediation Session – 21<sup>st</sup> Annual International conference on Soils, Sediments, and Water. Oct 17-20, Amherst MA 2005.

Reynolds, C.M, D.B. Ringelberg, S.L. Larson, and N.R. Adrian. 2005. RDX Humification Potential in Surface Soils and Mineralization Immobilization Turnover. 2nd Sustainable Range Management Conference & Exhibition, 22-25 August, San Antonio, TX.

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### ***Other Technology Transfer Opportunities***

We have been approached by Dr. Thomas Spriggs of the consulting firm CH2MHill as to the feasibility of using this technology in the field, and to determine if we would be willing to help deploy the technology at a naval firing range in Vieques, Puerto Rico. We are still in discussion with him on this possibility.