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Capacities of Candidate Herbaceous Plants for Phytoremediation of Soil-based TNT and RDX on Ranges

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Final report

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Abstract: This report describes a study to quantify the phytoextraction and phytostabilization capacities of TNT and RDX from spiked soil in selected herbaceous species, while paying attention to storage and quality of breakdown products in vegetative plant parts. Ten plant species were included in the experiments. Dose-response experiments formed the basis for evaluating the uptake and tentative in-plant degradation of the soil-based energetics and biomass characteristics of the plants. In these experiments, plants were exposed for periods ranging from 55 to 83 days in the greenhouse, biomass and evapotranspiration characteristics were determined, and residues of explosives' parent compounds and metabolites were analyzed using HPLC techniques.

Of the ten plant species tested, two grasses and four forbs were classified as TNT-tolerant. Total TNT loss from soil by processes other than plant TNT uptake ranged from 18.4 to 33.2 kg TNT ha⁻¹ in grasses and forbs, respectively. Plant TNT uptake ranged from 0.2 kg ha⁻¹ in grasses to almost none in forbs. Four grasses took up and metabolized TNT, and one forb showed some potential for TNT uptake and metabolism. All plant species were classified as RDX-tolerant. Total RDX loss from soil by processes other than plant RDX uptake ranged from 8.2 to 437 kg RDX ha⁻¹ in grasses and forbs, respectively. Plant RDX uptake ranged from 3.4 kg ha⁻¹ in grasses to 6.4 kg ha⁻¹ in forbs. Four grasses and one forb metabolized RDX. Two plant species were recommended for further exploration of their phytoextraction/plant-assisted phytoremediation capacity, both species of the uptaker/degrader type. Three other species were recommended for further exploration of their phytostabilization/plant-assisted phytoremediation capacity.

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Preface

This report was prepared by the U.S. Army Engineer Research and Development Center (ERDC), Environmental Laboratory (EL), Vicksburg, MS, in partnership with ERDC, Construction Engineering Research Laboratory (CERL), Champaign, IL; and the University of Illinois. The research was sponsored by the Strategic Environmental Research and Development Program (SERDP), Arlington, VA, Bradley P. Smith, Executive Director, and Dr. Jeff Marqusee, Technical Director, under Environmental Restoration Project Number ER1500. The principal investigator was Dr. Elly P. H. Best, Environmental Risk Assessment Branch (ERAB), Environmental Processes and Engineering Division (EPED), EL. Co-principal investigator was Thomas Smith, Ecological Processes Branch, Installations Division (CN), CERL.

The phytoremediation capacity studies were conducted: (a) for grass species by Dr. Best and Alan Torrey, at ERDC-EL, Vicksburg; and (b) for forb species by Thomas Smith, Frank Hagen, ERDC-CERL, and Dr. Jeffrey O. Dawson, University of Illinois, Urbana-Champaign, IL.

This report was reviewed by Dr. Fiona Crocker, EPED, ERDC-EL, and Dr. Dick Gebhart, CN, ERDC-CERL. The study was conducted under the direct supervision of Dr. Richard E. Price, Chief, EPED, EL; Dr. Beth C. Fleming, Director, ERDC-EL; Alan B. Anderson, Chief, EPB, ERDC-CERL; and Dr. Ilker Adiguzel, Director, ERDC-CERL.

COL Gary E. Johnston was Commander and Executive Director of ERDC. Dr. James R. Houston was Director.

1 Introduction

Military ranges and contamination by energetics

Military training ranges are important to the readiness of the Army and Department of Defense. A recent suspension of military activities at the Massachusetts Military Reservation (MMR) has alerted managers at all ranges to carefully assess their environmental status. The military mission requires that vegetation, largely composed of grasses, be as resilient as possible to military training exercises to maintain realism and control erosion. Major concerns are the mobility of energetics residues, and contamination of soils and groundwater. Explosives residues, such as 2,4,6-trinitrofluorene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX); and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX); on military ranges has been documented recently (from 1999 onwards; Pennington et al. 2001, 2003; Clausen et al. 2004; Efrogmson et al. in press) in the United States and in Canada. Contamination threats on ranges include leaching into groundwater, dissolution into groundwater, dissolution and flow into surface water, direct contact, and plant uptake and introduction into the food chain. The components of an ecosystem, such as its vegetative cover and soil types and their proximities to surface waters, play important roles in determining potential contaminant pathways on a particular range. Possible movement pathways of contaminants are through soil leaching and plant uptake.

Toxicity of energetics to plants

Among energetics, TNT and RDX are most widely distributed, and both compounds are often found in the soil at the same site. TNT is largely bound in soils, is leached in soils to a very low extent, and is taken up by plants. RDX has a high potential for soil leaching and can also be taken up by plants (Best et al. 1999). Published studies indicate that containment of both compounds in the vegetation can be substantial, and that degradation within plants is relatively low. A few studies of the phytotoxicity of energetics have already been published. Most of these, which are reviewed in Rocheleau et al. (2003), were tests of TNT. A limited number of studies on RDX and HMX, e.g., Schnoor et al. (2006), suggest that nitro-heterocyclic compounds are not as toxic as nitroaromatic compounds such as TNT. The published screening benchmark for TNT in soil for terrestrial plants is

30 mg kg⁻¹ (Talmage et al. 1999). This study is based on the Lowest Observed Effective Concentration (LOEC) of 30 mg TNT kg⁻¹ for aged soil, with a No Observed Effect Concentration (NOEC) of 10 mg TNT kg⁻¹ in bush bean (*Phaseolus vulgaris*; grass; Cataldo et al. 1989). More recently other phytotoxic concentrations have been published also. The published screening benchmark for RDX in soil for terrestrial plants is 100 mg kg⁻¹ (Talmage et al. 1999). This value is based on the LOEC of 100 mg RDX kg⁻¹ for aged soil in cucumber (*Cucumis sativa*; Simini et al. 1995). However, a concentration of >1,540 mg RDX kg⁻¹ soil failed to reduce the biomass of perennial ryegrass (*Lolium perenne*) and alfalfa (*Medicago sativa*) by 20 percent as required for a LOEC (Best et al. 2006). A screening benchmark for HMX has not been published.

Phytoremediation

Promising in situ technologies for contaminated soils include phytoextraction—the use of plants to take up (accumulate) and remove contaminants from the soil—and phytostabilization—the use of both plants and soil amendments to prevent the contaminants from migrating from the source area. Either phytoextraction or phytostabilization or a combination of both would be cost-effective, aesthetically pleasing, and not disruptive of range use, but the fate and transport characteristics of energetics in vegetated soils must be understood before phytoremediation can be effectively used with confidence.

Identification of candidate herbaceous plants for phytoremediation of energetics on ranges

In a recent study, rapidly colonizing and resilient grasses/forbs that are tolerant towards range-relevant contaminants, with emphasis on TNT and RDX, were identified (Best et al. 2007). First, herbaceous plant species with characteristics that make them potential candidates for use on ranges for phytostabilization and phytoextraction purposes were reviewed. This review was limited to native and introduced grass and forb species, and species with improved genetic characteristics that have successfully been used on training lands in North America. The eight criteria used to select plant species for short-term screening experiments included (1) tolerance towards energetics, (2) resilience-related life cycle characteristics and plant traits, (3) typical biogeographic distribution, (4) seed size, (5) availability of propagules, (6) photosynthetic pathway, (7) exceptional traits, and (8) other. Subsequently, eight grasses and eight forbs were selected

from the reviewed species for short-term tolerance testing, among which five grasses and five forbs showed considerable tolerance towards TNT and RDX. The latter ten species were recommended for further evaluation of their phytoremediation potential. The geographic distribution and expected occurrence of the screened plant species are provided in Appendix A.

Objectives

The objectives of the current study were to quantify energetics (TNT, RDX, with RDX emphasized) phytoextraction and phytostabilization capacities in energetics-tolerant herbaceous plants, while paying attention to storage and quality of breakdown products in vegetative plant parts. The ten plant species identified as short-term tolerant to TNT and RDX were used in this study.

2 Materials and Methods

Energetics chemicals and standards

Technical grade TNT and RDX were obtained from the Central Explosives Holding Area, Waterways Experiment Station, Vicksburg, MS. The technical TNT was purified by four successive recrystallization cycles in methanol at 40 °C. Verification of the purity of TNT using HPLC analysis indicated 1 percent TNB. The technical RDX was purified by two successive recrystallization cycles in water at 100 °C. Verification of RDX using HPLC analysis indicated 4 percent HMX. The purities were considered appropriate for metabolic studies. Energetics standards were purchased from Accu Standard Inc., Ellington, CT.

Experimental

Dose-response curves for concentrations between 0 and 100 mg TNT kg⁻¹ dry weight (DW), and between 0 and 1,000 mg RDX kg⁻¹ DW were constructed for the plant tests. The test substrates were prepared by spiking with different volumes of the same methanolic stock solution. Non-spiked soil served as a control. All treatments were replicated seven times, and treatments followed a randomized block design for each plant group (grasses and forbs). The studies included a total of 245 test units each per plant group [(1 control × 5 species × 7 replicates) + (3 TNT treatments × 5 species × 7 replicates) + (3 RDX treatments × 5 species × 7 replicates)].

The following parameters were determined:

- In plants as a basis for the evaluation of the plant response
 - Biomass characteristics (above- and belowground biomass, root length, root surface area, root diameter) at the end of the cultivation period
 - Evapotranspiration characteristics (grasses only)
 - Concentrations of energetics and metabolites
- In soils as a basis for the evaluation of soil-based energetics remediation
 - Concentrations of energetics compounds and metabolites initially and at the end of the cultivation period

Cultivation periods were used that were long enough to allow the plants to reach maturity, and, thus, to evaluate plant persistence potential at the soil-based energetics levels tested. These periods varied with plant species: 61 days for the grasses, 55 days for *A. retroflexus*, 56 days for *I. lacunosa*, 70 days for *A. syriaca*, 76 days for *P. oleracea*, and 83 days for *S. spinosa*.

Soil

Camp Shelby is located in Perry County, MS, near the town of Hattiesburg. The distribution of soils in Camp Shelby was determined from the *Soil Survey of Perry County, Mississippi* (Daniels 1999). About 80 percent of the operational area of Camp Shelby is in the DeSoto National Forest in Perry County. Camp Shelby is a training and mobilization facility for National Guard units. About 30 different soils occur in the county, which range widely in texture, natural drainage, slope, and other characteristics. The appearance of the soil, excavated for the experiments, agreed with the description of the McLaurin-Benndale-Smithdale association, characterized as “dominantly nearly level to strongly sloping, well-drained loamy soils weathered from unconsolidated loamy sediments.” It agreed most with the McLaurin characteristics, i.e., surface layer dark grayish brown fine sandy loam; subsurface layer yellowish brown, fine, sandy loam; subsoil upper part-yellowish red sandy loam with red mottles; lower part—red sandy loam; well-drained. Soil was collected from the primary soil site in the northern part of Camp Shelby. This site was selected because it was sparsely vegetated by pine trees and herbaceous vegetation, regularly used for surface soil excavation, and easily accessible (Figure 1). Vegetation and surficial detritus were removed, surface soil up to a depth of 30 cm was excavated using a backhoe, and all were transferred to the back of a truck (Figure 2). The soil was transported to the University of Illinois, Champaign, for further processing. The soil was air-dried in a vented greenhouse, and mixed. The soil was passed through an M-4 hammer mill shredder (Lindig Mfg. Corp., St. Paul, MN) to ensure homogeneous water penetration of soil when irrigated in the laboratory (Figure 3).

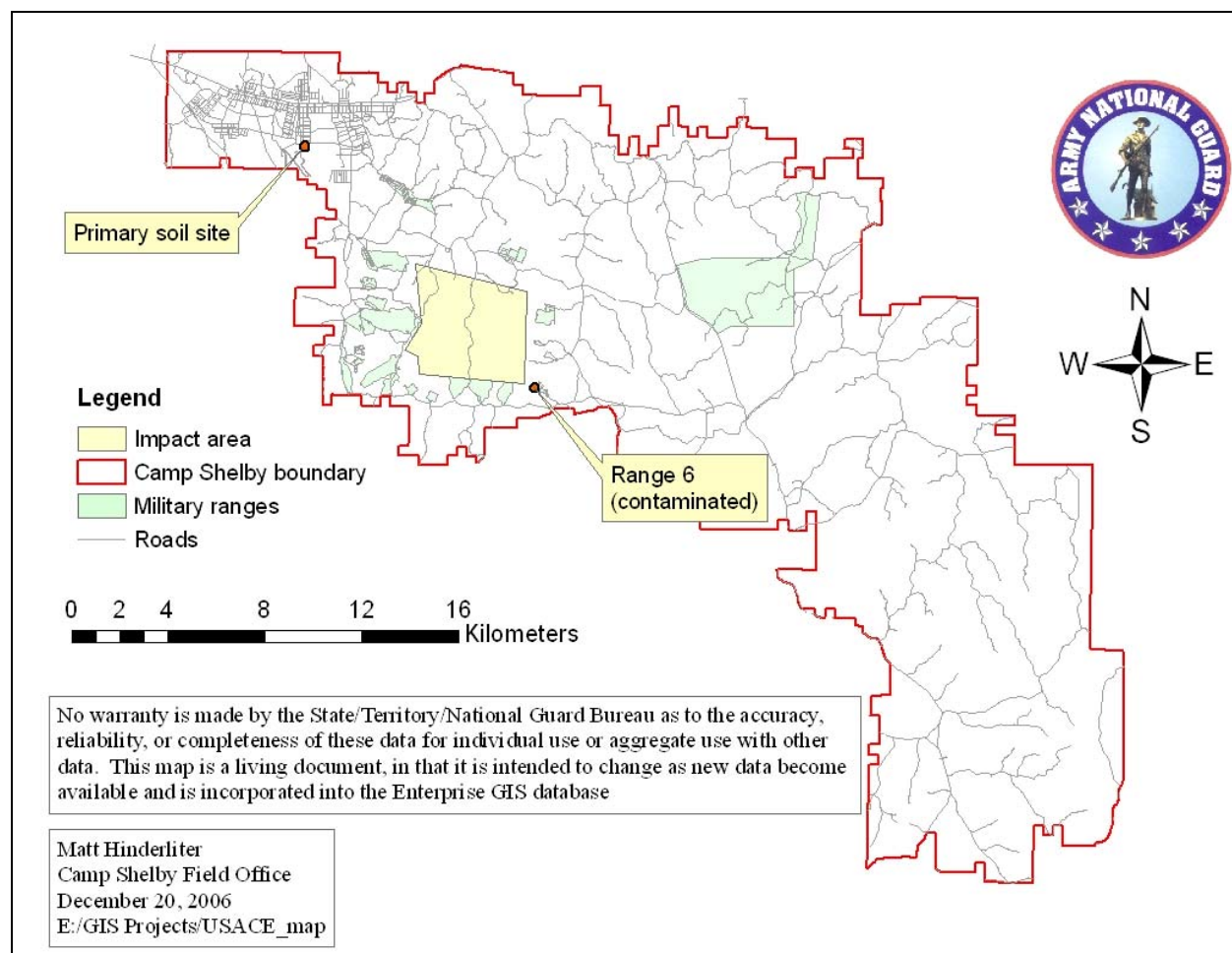


Figure 1. Location of soil excavation site within Camp Shelby, MS.

This soil was used for the pot experiments, for the grass experiment in Vicksburg, MS, after transport in 190-L barrels, and for the forb experiment in Champaign, IL, directly.

For the TNT tests, the soils were sprayed with 10, 50, and 100 mg TNT kg⁻¹ DW using methanol as a solvent. For the RDX tests, the soils were sprayed with 100, 500, and 1,000 mg RDX kg⁻¹ DW. After spraying, the soils were mixed with a stainless-steel scoop, and placed in a vented greenhouse without illumination overnight to allow the methanol to evaporate prior to exposure of the test organisms.



Figure 2. Collecting soil from Camp Shelby, MS.

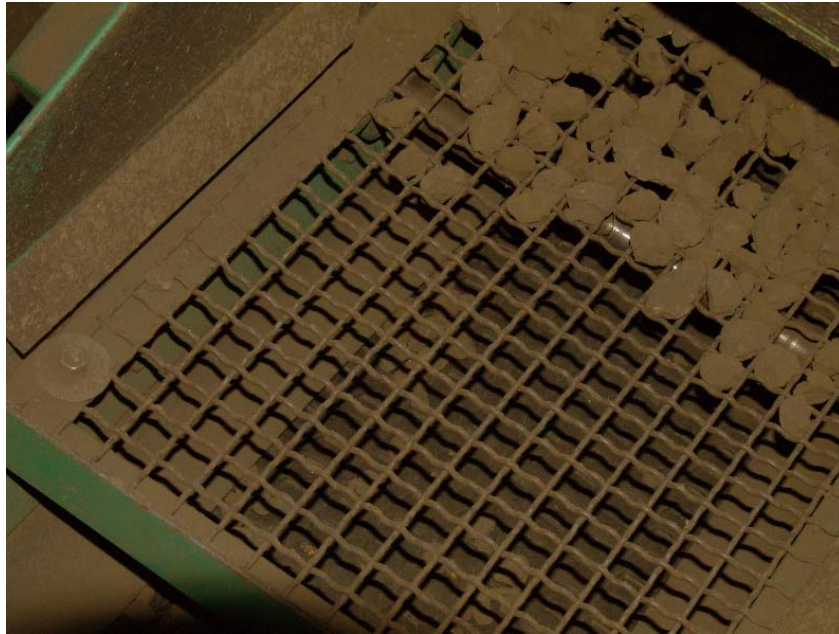


Figure 3. Grinding soils using an M-4 hammer mill shredder.

Plant materials

Propagules of the ten plant species previously identified as tentative candidates for inclusion in the energetics phytoremediation experiments (Best et al. 2007) were purchased as follows:

- Grass seeds of big bluestem (*Andropogon gerardii*), blue grama (*Bouteloua gracilis*), Canadian wild rye (*Elymus canadensis*), sand lovegrass (*Eragrostis trichoides*), and Indiangrass (*Sorghastrum nutans*) from the Granite Seed Company, Lehi, UT
- Forb seeds from three vendors as follows: redroot pigweed (*Amaranthus retroflexus*), morning glory (*Ipomoea lacunosa*), and prickly sida (*Sida spinosa*) from Azlin Seed Service, Leland, MS; common milkweed (*Asclepias syriaca*) from Prairiemoon Nursery, Winona, WI; common purslane (*Portulaca oleracea*) from Monsanto Seed Library, St. Louis, MO

Plant exposures, plant harvesting, and sample preparation of plants and soils

For each unit, a weight equivalent to 200 seeds was placed on top of air-dry soil equivalent with 768 g DW of the appropriate soil mixture, contained in 2-L plastic pots. The grass seeds were seeded directly after weighing, and the forb seeds were seeded after overnight soaking in

0.5 mg L⁻¹ gibberellic acid to enable rapid, synchronized germination. The pots were covered with transparent plastic lids and sprayed with reverse osmosis (RO) water immediately after placing the test seeds on the soils, and, subsequently, every day as needed until seedlings were visible. Subsequently, the pots were watered twice a week with RO water to maintain the soil at a moisture level of 36 percent (field capacity was 38 percent). A moisture level at field capacity allows maximum mobility of contaminants in soil solution. Plants were amended with slow-release Osmocote fertilizer 10 days after the onset of the experiment to attain target levels of 352 kg N ha⁻¹, 59.2 kg P ha⁻¹ and 331.9 kg K ha⁻¹, commonly used for pastures (Best and Jacobs 2001). Seeds germinated synchronously, as was verified before the onset of the tests. Plants were cultivated as follows: (1) the grasses in a greenhouse of the Environmental Laboratory, Vicksburg, MS, and (2) the forbs in a greenhouse of the University of Illinois, Urbana-Champaign, Champaign, IL. For grasses, the tests lasted from 21 April to 20 June 2007 (61 days; Figure 4). For forbs, the tests lasted from 6 June to 28 August 2007 (55–83 days).

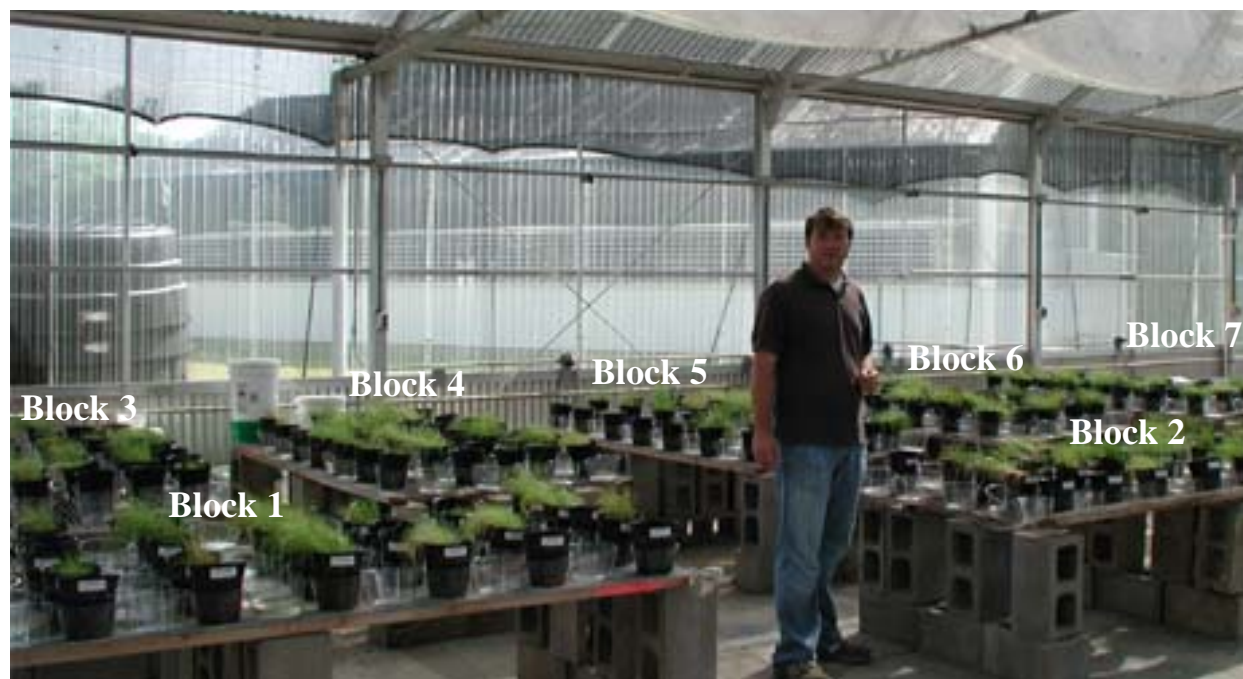


Figure 4. The grass and forb experiments both followed a randomized block design, in seven blocks. All treatments were replicated seven times. Grass experiment was conducted in a greenhouse at the Environmental Laboratory in Vicksburg, MS.

At the end of the cultivation periods the plants were harvested in preparation for tissue analysis for explosives. Above- and belowground plant portions were separated using stainless-steel scissors. The plant tissues

were washed in RO water to remove dust and soil particles, blotted as dry as possible, and weighed. After collecting, washing, blotting, and weighing was completed, plant tissues were placed in plastic Ziploc bags and frozen at $-80\text{ }^{\circ}\text{C}$. Subsamples were used to determine dry weight. Dry weight was determined by drying the fresh material in a forced-air oven to constant weight ($105\text{ }^{\circ}\text{C}$). To determine energetics in plant tissues and soil, modifications of Method 8330 for soils (USEPA 1992) were used, as described below.

Extractions and energetics analyses

Plant extracts were prepared from freshly ground materials. Soil extracts were prepared from air-dry material. Only three of the seven replicate samples of each treatment were extracted. This was done because variations in biomass were expected to be larger than those in explosives concentrations, and to limit analytical costs.

Plants were clipped into small pieces and mixed. Subsamples for extraction were homogenized by grinding them in liquid nitrogen. Two-gram fresh weight (FW) portions were spiked with 1,3-dinitrobenzene (1,3-DNB) as internal standard for recovery ($50\text{ }\mu\text{L}$ of a 1 mg mL^{-1} solution), and extracted in 5-mL acetonitrile by an 18-h sonication in a water-cooled bath at $15\text{ }^{\circ}\text{C}$. The extracts were freed from particles by centrifugation for 10 min at 2,000 g. Then 2-mL aliquots of the supernatants were cleaned over a 0.5-g Florisil solid phase extraction (SPE) column, concentrated 10x by evaporation under a stream of N_2 at $35\text{ }^{\circ}\text{C}$, and the final sample volume was adjusted to 1.5 mL with 1:1 acetonitrile: Millipore-filtered RO water. The samples were freed from remaining particles by cleanup over a $0.45\text{-}\mu\text{m}$ polytetrafluoroethylene (PTFE) disk.

Just before incubation, each soil mixture was analyzed for energetics and other chemical and physical characteristics, in triplicate. At the end of the incubation, three replicates of each treatment, corresponding to the analyzed plant replicates, were extracted and analyzed for energetics residues. The 2-g air-dry weight portion was extracted in 5 mL acetonitrile by 18-h sonication at $15\text{ }^{\circ}\text{C}$, cleanup over a Florisil column, 10x concentration, and cleanup over a PTFE disk.

High performance liquid chromatography (HPLC) analysis of the final extracts was carried out. The plant and soil extracts of the samples in which the highest energetics levels were expected were first screened for

the presence of all compounds listed by Method 8330 (USEPA 1992). After identifying the energetics' parent compounds and metabolites in these extracts, only the relevant compounds were determined in all other extracts. The latter compounds were usually 2,4,6-trinitrotoluene (TNT), 2-amino-4,6-dinitrotoluene (2-ADNT), 4-amino-2,6-dinitrotoluene (4-ADNT), hexahydro-1,3,5-trinitro-1,3,5 triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7- tetrazocine (HMX), and (1,3-DNB) as internal standard.

The method detection level (MDL) in mg kg⁻¹ DW for several target compounds, spiked on plants and soil directly before extraction, varied with compound.

- In freshly ground plant tissues

MDL: TNT 0.081, 2-ADNT 0.103, 4-ADNT 0.161, 4-nitrotoluene (4-NT) 0.314, RDX 0.142, HMX 0.110 mg kg⁻¹ DW

- In air-dry ground soil

MDL: TNT 0.1684, 2-ADNT 0.3043, 4-ADNT 0.1225, RDX 0.3122, HMX 0.1913 mg kg⁻¹ DW

Recovery of 1,3-DNB was usually 95 percent.

Characterization root systems

The root systems of all plants were characterized by determination of the total length (m), average diameter (mm), and surface area (m²) using a WinRHIZO system (WinRHIZO Pro LA2400; Regent Instruments Inc., Quebec, Canada). For these determinations, subsamples of the washed root systems of a known weight were spread as homogeneously as possible in a translucent tray, black and white images were collected using an Epson LA2400 scanner equipped with a back-lighting source (EPSON Expression 10000XL 1.0 TWAIN source), and images were analyzed using the WinRHIZO Pro software package (Figure 5). Because the surface area:weight ratio differed considerably among plant species, the weights of the root subsamples subjected to the scanning procedure were selected to ensure a standard deviation of 2 percent for three subsamples of the same root system (range 0.2-0.4 g FW).

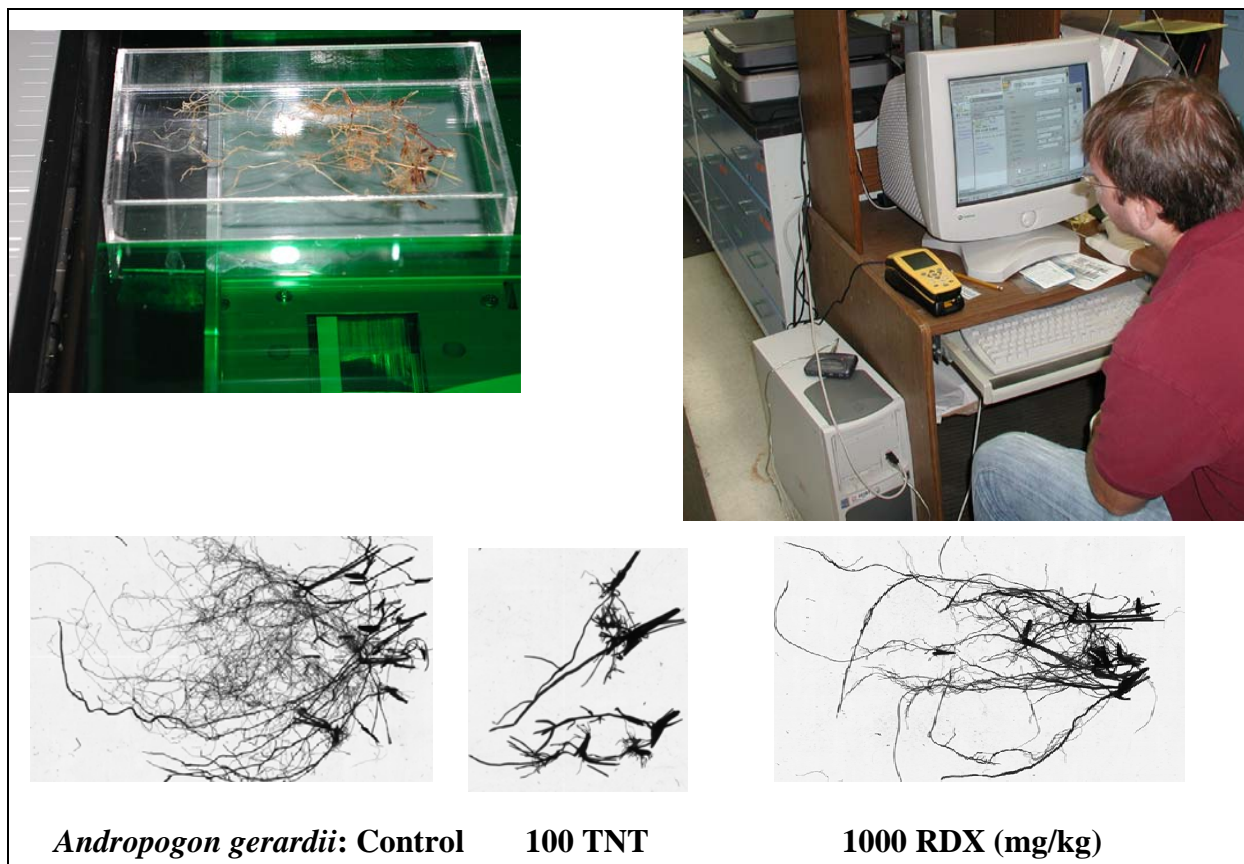


Figure 5. Characterization of the root systems using a WinRHIZO system. Subsamples of washed root systems were spread in a translucent tray, black and white images were collected using a scanner, and images were analyzed using the WinRHIZO pro software package (upper). Images typical for control roots and roots exposed to TNT and RDX, respectively, are shown (lower).

Evapotranspiration estimates

Evapotranspiration rates were measured in the grasses by weekly weighing of one replicate per treatment and recording the water volumes added. Evapotranspiration rates per pot were scaled up to represent vegetation covering 1 m². This method quantifies plant transpiration and soil evaporation together and provides information on evapotranspiration of 1-m² well-drained loamy soil covered by the grass species used in the current study. Evapotranspiration rates in forbs have not been measured.

Other soil analyses

Moisture content was determined by drying at 105 °C in a forced-air oven until constant weight. Concentrations of organic matter were determined by loss on ignition at 550 °C, and bulk density volumetrically (Allen et al. 1974). The pH, nutrients, basic cations, and cation exchange capacity (CEC) were determined according to *Recommended Chemical Soil Test*

Procedures for the North Central Region (of the United States). The pH was determined in a 1:1 soil:water buffer solution, consisting of 37 g KCl, 215.25 g KOH, 10 g nitrophenol, and 7.5 g boric acid per L of water (Watson and Brown 1998). Phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) were determined by atomic absorption spectrophotometry in malic acid extracts; phosphorus according to Frank et al. (1998), and K and other basic cations according to Warncke and Brown (1998). The CEC was determined mathematically from the values for P, K, Ca, Mg, and pH. The results of these analyses are presented in Table 1.

Table 1. Properties of clean¹ Camp Shelby soil prior to the amendments and tests.

Property	Level
Nutrients and Ions	
Total Phosphorus (mg kg ⁻¹ DW)	3.99 ± 1.07
Potassium (mg kg ⁻¹ DW)	47.15 ± 4.67
Calcium (mg kg ⁻¹ DW)	368.85 ± 26.08
Magnesium (mg kg ⁻¹ DW)	79.56 ± 5.80
Hydrogen [meq (100 g) ⁻¹ DW]	47.84 ± 2.06
Other	
pH Water	4.70 ± 0.10
Organic Matter (%DW)	2.48 ± 0.03
Dry Weight (%FW)	96.48 ± 0.4
Bulk Density (g DW mL ⁻¹)	1.61 ± 0.01
Cation Exchange Capacity [meq (100 g) ⁻¹ DW]	4.85 ± 0.16
Note: Mean values and standard deviations (N=3). DW: dry weight; FW: fresh weight.	
¹ This soil was not subjected to explosives and organics analyses since no prior history of exposure existed. Nitrogen was not determined because the soil was fertilized during cultivation.	

Data analysis

Statistical analyses were conducted with the software STATGRAPHICS Plus for Windows Version 32S package (Manugistics, Rockville, MD). Normal distribution of the data was tested using the Shapiro-Wilk's test.

Analysis of variance (ANOVA) was conducted and expanded in several cases with a multiple range test using the Fisher's least significant difference procedure. The p-value in the ANOVA is a measure of the significance of the analysis; it was set at a 95 percent confidence levels (p-value of ≤0.05). In this analysis the sum of plant tissue 2-ADNT and 4-ADNT

concentrations was included as TNT-equivalents (recalculated on a molar basis). The plant tissue mononitroso-RDX (MNX), dinitroso-RDX (DNX), and trinitroso-RDX (TNX) concentrations were not included in the statistical analyses.

Linear regression analyses were conducted using the least squares method. Non-linear equations were fitted with the polynomial regression module using the least squares method. The p-value in the regression model was set at a 95-percent confidence level (p-value of ≤ 0.05) unless stated otherwise. The R^2 -value of the regression model indicates the proportion of the variance explained by the model. Regression models explaining at least 50 percent of the variability in the data set, i.e., $R^2 \geq 0.50$, were considered as meaningful.

3 Results

Plant response to soil-based energetics treatment

TNT exposures

Grasses

Plant biomass production of the grasses included in the experiment was significantly affected by TNT concentration ($p < 0.001$), species ($p < 0.001$), and by their interaction ($p < 0.001$; Table 2). The block effects were not statistically significant ($p = 0.937$); therefore, all data were analyzed as if completely randomized. Because the interaction term was significant, the overall TNT exposure effect could not be separated from the species effect.

Table 2. Plant biomass of grasses in response to 61 days of exposure to TNT-contaminated soil. Mean values and standard deviations are shown (N=7). Values that are followed by the same letter are not significantly different according to Fisher's least significant difference procedure. ANOVA¹ results are listed.

TNT Exposure Grasses			
Species	Plant Biomass (g DW m ⁻²)		
<i>A. gerardii</i>	301.89 (166.32) c		
<i>B. gracilis</i>	21.81 (8.87) a		
<i>E. canadensis</i>	76.13 (46.54) b		
<i>E. trichoides</i>	6.78 (10.22) a		
<i>S. nutans</i>	341.78 (182.91) d		
ANOVA ¹			
Factor	MS	F-ratio	p-value
TNT-exposure	255,473.0	166.32	<0.001
Species No.	709,778.0	462.09	<0.001
TNT-exposure x Species No.	63,374.3	41.26	<0.001
¹ ANOVA results of plant biomass data, using target explosives concentration, species, and their interaction as factors (species entered as number in the analysis). Underlining marks a statistically significant effect.			

As shown in Table 2 and Figure 6, plant biomass production varied greatly with species, being very low in *B. gracilis* and *E. trichoides*, intermediate in *E. canadensis*, and significantly greater in *A. gerardii* and *S. nutans*.

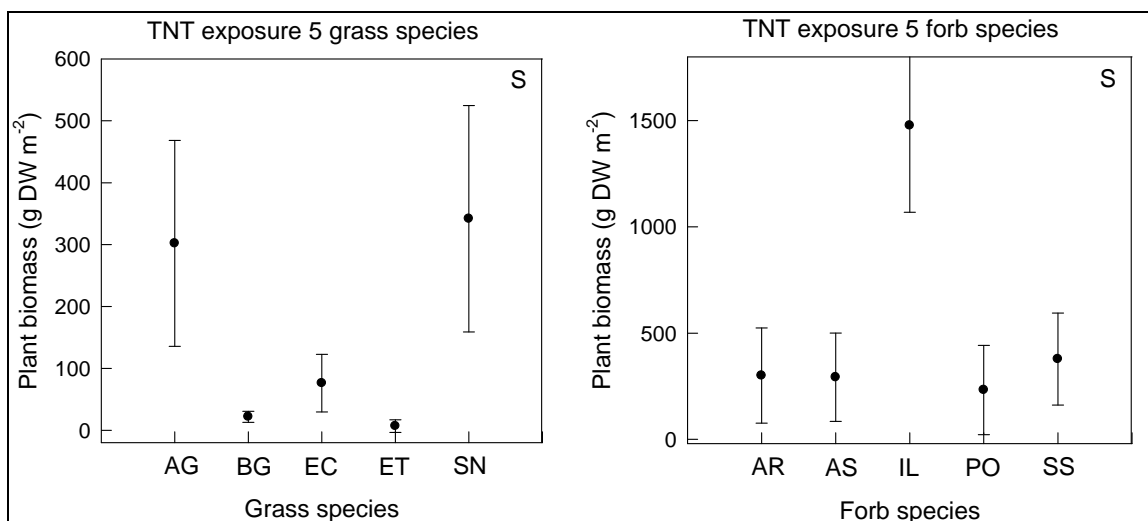


Figure 6. Biomass of plants in response to 61 days of exposure to TNT-contaminated soil. Mean values and standard deviations. Abbreviations: AG - *A. gerardii*; BG - *B. gracilis*; EC - *E. canadensis*; ET - *E. trichoides*; SN - *S. nutans*; AR - *A. retroflexus*; AS - *A. Syriaca*; IL - *I. Lacunosa*; PO - *P. oleracea*; SS - *S. spinosa*; S is statistically significant.

Plant biomass production was significantly affected by TNT exposure in all grasses (Table 3). Plant biomass usually decreased with increasing soil TNT concentration up to 100 mg kg⁻¹ soil, except in *A. gerardii* where 10 mg kg⁻¹ soil stimulated plant biomass (Table 3, Figures 7 and 8). Root production was inhibited at lower soil TNT levels than shoot production.

Table 3. Plant biomass of individual grass species in response to 61 days of exposure to TNT-contaminated soil. Mean values and standard deviations are shown (N=7). Values that are followed by the same letter are not significantly different according to Fisher's least significant difference procedure. ANOVA¹ results are listed.

TNT Exposure Grasses					
Factor	Plant Biomass (g DW m ²)				
TNT Exposure	<i>A. gerardii</i>	<i>B. gracilis</i>	<i>E. canadensis</i>	<i>E. trichoides</i>	<i>S. nutans</i>
Control	353.63 (39.58) b	31.18 (9.55) c	116.78 (16.00) c	9.93 (7.07) b	454.65 (63.51) b
10 mg kg ⁻¹ TNT	488.24 (45.97) c	25.22 (5.43) b	114.02 (16.07) c	17.64 (12.50) b	449.38 (137.62) b
50 mg kg ⁻¹ TNT	317.34 (29.38) b	16.16 (3.14) a	61.91 (23.64) b	0 a	405.20 (19.33) b
100 mg kg ⁻¹ TNT	48.33 (32.12) a	14.69 (3.29) a	11.80 (3.00) a	0 a	57.90 (11.12) a
ANOVA ¹					
Factor	MS	F-ratio	p-value		
TNT-exposure AG	237,841.0	170.69	<0.001		
TNT-exposure BG	424.5	12.01	<0.001		
TNT-exposure EC	17,334.9	64.09	<0.001		
TNT-exposure ET	509.5	9.87	<0.001		
TNT-exposure SN	254,155.0	43.32	<0.001		

¹ ANOVA results of plant biomass data, using target explosives concentration as factor. Underlining marks a statistically significant effect.

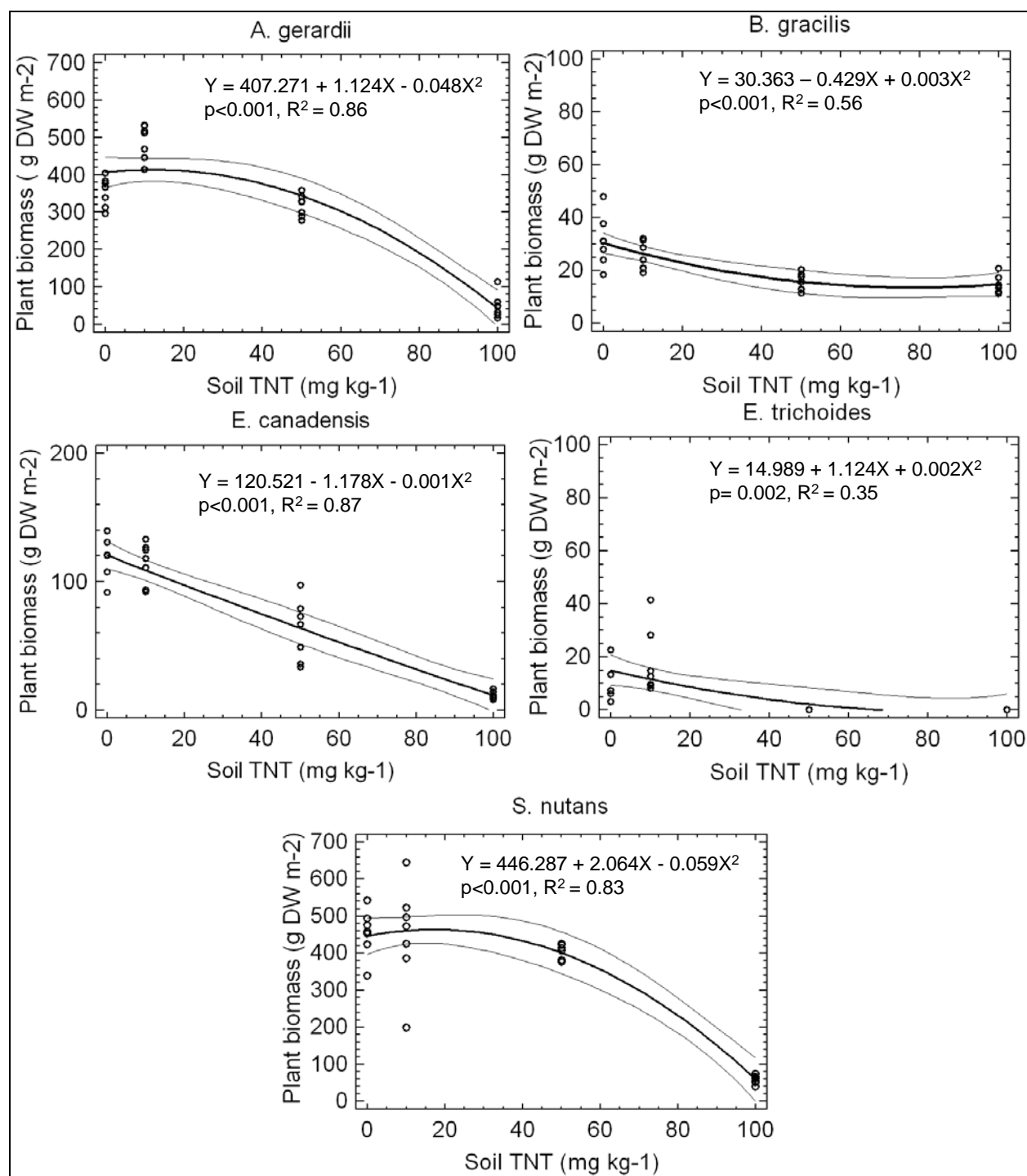


Figure 7. Plant biomass of individual grass species in response to 61 days of exposure to TNT-contaminated soil. Regression lines and 95% confidence limits indicated; Y = plant response, X = target explosives concentration soil mixture.

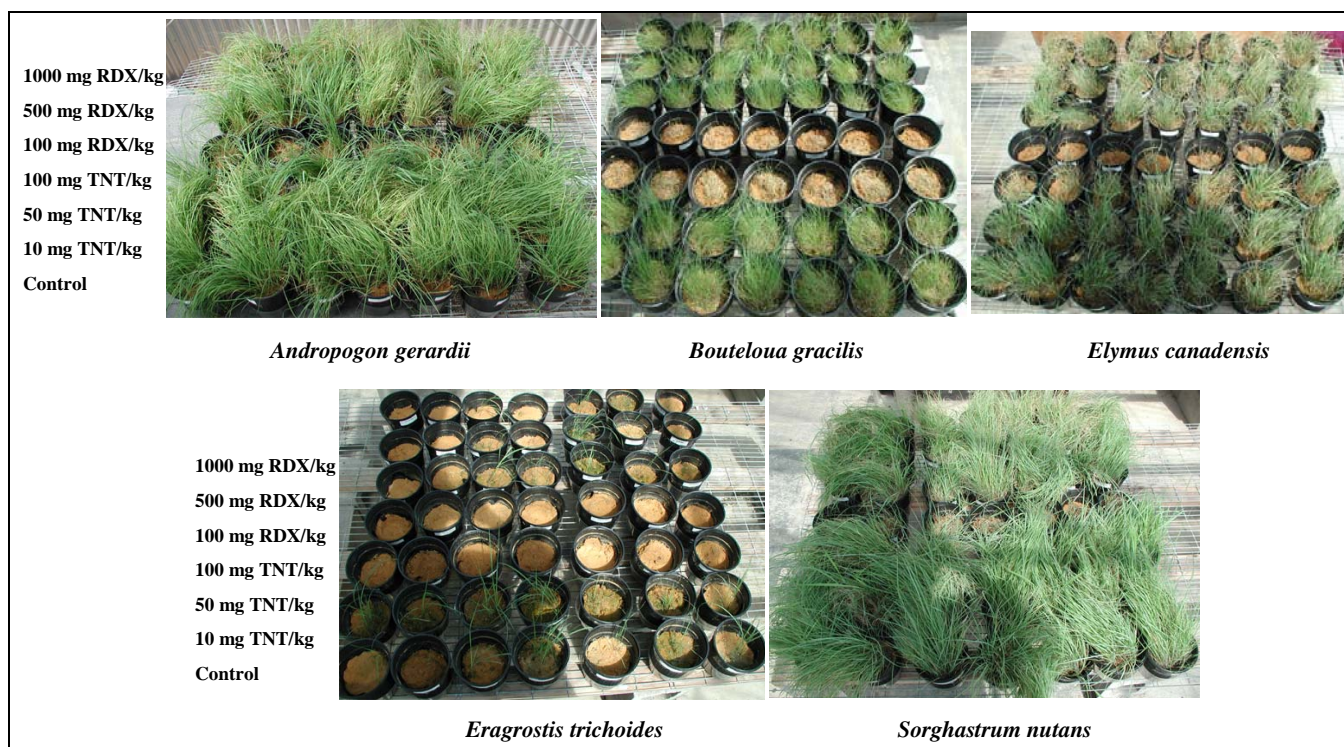


Figure 8. Typical responses of five grass species to 61 days of exposure to explosives-contaminated soils.

Root production was completely prevented in *B. gracilis* at a soil TNT level >10 mg kg⁻¹, and in *E. canadensis* and *S. nutans* at a soil TNT level >50 mg kg⁻¹, with all these plants producing shoots (Tables 3 and 4). Overall germination in *E. trichoides* was very poor, and, therefore, these plants were only analyzed for selected parameters. Both root and shoot formation were prevented at a soil TNT level >10 mg kg⁻¹ in the latter species (Table 3).

Based on the criterium of having a healthy appearance, i.e., showing a vigorous, green appearance, and in the possession of well-developed roots, the following grass species x treatment combinations were characterized as “healthy”:

- *A. gerardii*, control, 10 mg, 50 mg TNT kg⁻¹ soil
- *B. gracilis*, control, 10 mg TNT kg⁻¹ soil
- *E. canadensis*, control, 10 mg TNT kg⁻¹ soil
- *S. nutans*, control, 10 mg, 50 mg TNT kg⁻¹ soil

Table 4. Shoot:root ratio, root characteristics, and evapotranspiration rates of individual grass species in response to 61 days of exposure to TNT-contaminated soil. Mean values and standard deviations are shown (N=7). A = absent.

TNT Exposure Grasses					
TNT Exposure	S:R Ratio	Root Characteristics			Evapotranspiration (L m ⁻² d ⁻¹)
		Length (m g ⁻¹ DW)	Diameter (mm)	Surface Area (m ² g ⁻¹ DW)	
<i>A. gerardii</i>					
Control	1.8 (0.3)	99.8 (19.6)	0.24 (0.02)	0.07 (0.01)	2.40
10 mg kg ⁻¹ TNT	2.0 (0.3)	76.0 (13.2)	0.27 (0.02)	0.07 (0.01)	2.60
50 mg kg ⁻¹ TNT	2.4 (0.4)	40.3 (14.0)	0.38 (0.08)	0.05 (0.01)	2.50
100 mg kg ⁻¹ TNT	1.3 (0.5)	26.0 (6.1)	0.51 (0.04)	0.04 (0.01)	1.68
<i>B. gracilis</i>					
Control	5.5 (1.8)	325.8 (40.0)	0.32 (0.02)	0.32 (0.03)	1.40
10 mg kg ⁻¹ TNT	4.0 (0.5)	332.2 (60.5)	0.32 (0.03)	0.33 (0.04)	1.28
50 mg kg ⁻¹ TNT	A	A	A	A	1.34
100 mg kg ⁻¹ TNT	A	A	A	A	1.56
<i>E. canadensis</i>					
Control	1.7 (0.2)	112.1 (14.9)	0.26 (0.02)	0.09 (0.01)	2.14
10 mg kg ⁻¹ TNT	1.7 (0.3)	109.8 (22.4)	0.27 (0.03)	0.09 (0.01)	2.09
50 mg kg ⁻¹ TNT	39.7 (22.3)	55.6 (9.5)	0.41 (0.02)	0.07 (0.01)	1.43
100 mg kg ⁻¹ TNT	A	A	A	A	1.72
<i>E. trichoides</i>					
Control	3.9 (2.8)	29.2 (2.0)	0.24 (0.03)	0.02 (0.00)	1.69
10 mg kg ⁻¹ TNT	2.7 (2.2)	17.9 (7.8)	0.31 (0.10)	0.02 (0.00)	1.81
50 mg kg ⁻¹ TNT	A	A	A	A	1.95
100 mg kg ⁻¹ TNT	A	A	A	A	1.88
<i>S. nutans</i>					
Control	2.6 (0.6)	92.7 (24.6)	0.23 (0.02)	0.09 (0.01)	2.23
10 mg kg ⁻¹ TNT	2.7 (1.0)	77.5 (10.7)	0.23 (0.02)	0.06 (0.00)	2.23
50 mg kg ⁻¹ TNT	2.4 (0.4)	59.6 (15.1)	0.29 (0.04)	0.05 (0.01)	2.15
100 mg kg ⁻¹ TNT	A	A	A	A	1.69

The shoot:root (S:R) ratio in the grass controls was usually approximately two, indicating that twice as much biomass was produced aboveground as belowground on a dry weight basis (Table 4; Figure 9). Only in *B. gracilis* the S:R ratio was two times higher (Figure 10). In *A. gerardii*, the S:R ratio showed a slight increase upon exposure up to 50 mg TNT kg⁻¹ soil, followed by a decrease (Figure 9). In *E. canadensis*, the S:R ratio increased greatly upon exposure to 50 mg TNT kg⁻¹ soil because the plants had virtually no roots (Figure 9). In three grasses, i.e., *B. gracilis*, *E. trichoides*, and *S. nutans*, the S:R ratio did not exhibit a significant relationship with soil TNT level (Figure 10).

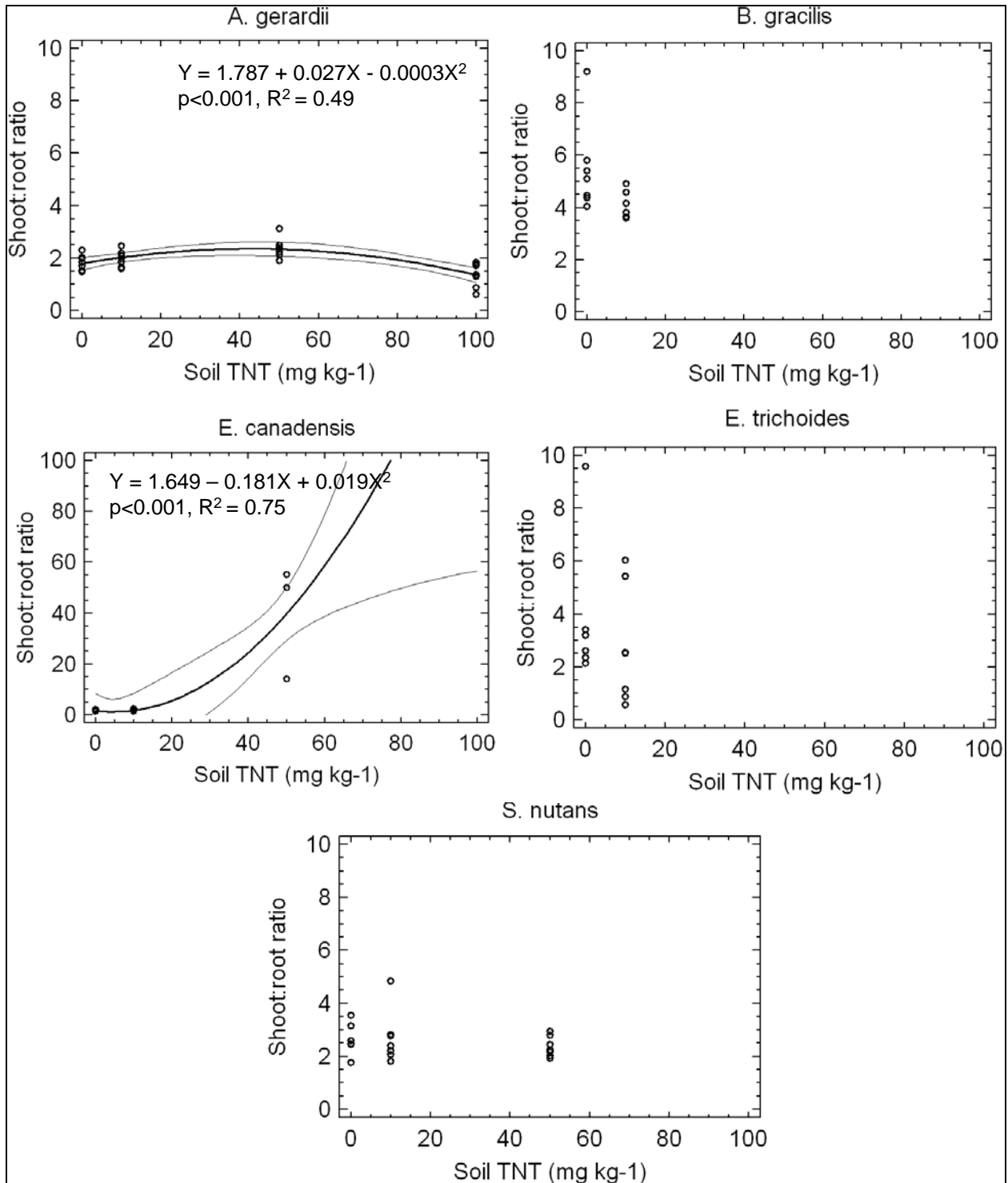


Figure 9. Shoot:root ratio of individual grass species in response to 61 days of exposure to TNT-contaminated soil. Regression lines and 95% confidence limits indicated where $p < 0.05$; Y = plant response, X = target explosives concentration soil mixture.

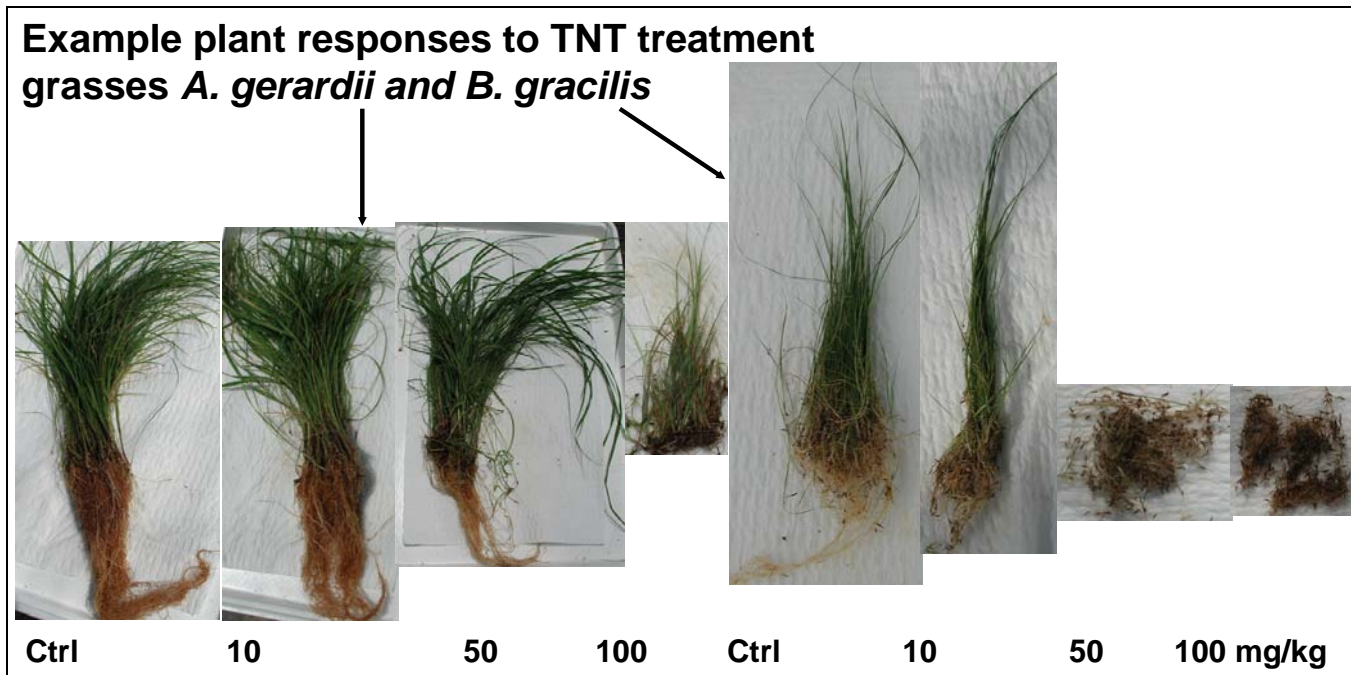


Figure 10. Typical response of two grass species to 61 days of exposure to TNT-contaminated soil illustrating significantly reduced root systems.

Root characteristics varied greatly with species. Specific root length was far greater in *B. gracilis* (326 m g⁻¹ DW in controls; Table 4) than in all other grasses. Root length was on the order of 100 m g⁻¹ DW in *A. gerardii*, *E. canadensis*, and *S. nutans* controls, and it was least in *E. trichoides* controls (on the order of 30 m g⁻¹ DW). Root diameter in control plants ranged from 0.23 to 0.32 mm (Table 4). Specific root length decreased and root diameter increased with soil TNT level, but these relationships were not significant in all cases (Table 4). Significant, linear, species-characteristic relationships between root diameter and specific root length were found, indicating critical combinations of maximum root diameter and maximum specific root length for grasses upon exposure to TNT. The following critical combinations were calculated using the regression equations (Figure 11):

- *A. gerardii*: Root diameter 0.59 mm, specific root length 148 m g⁻¹ DW
- *B. gracilis*: Root diameter 0.49 mm, specific root length 953 m g⁻¹ DW
- *E. canadensis*: Root diameter 0.53 mm, specific root length 222 m g⁻¹ DW
- *E. trichoides*: Root diameter 0.64 mm, specific root length 39 m g⁻¹ DW
- *S. nutans*: Root diameter 0.41 mm, specific root length 194 m g⁻¹ DW

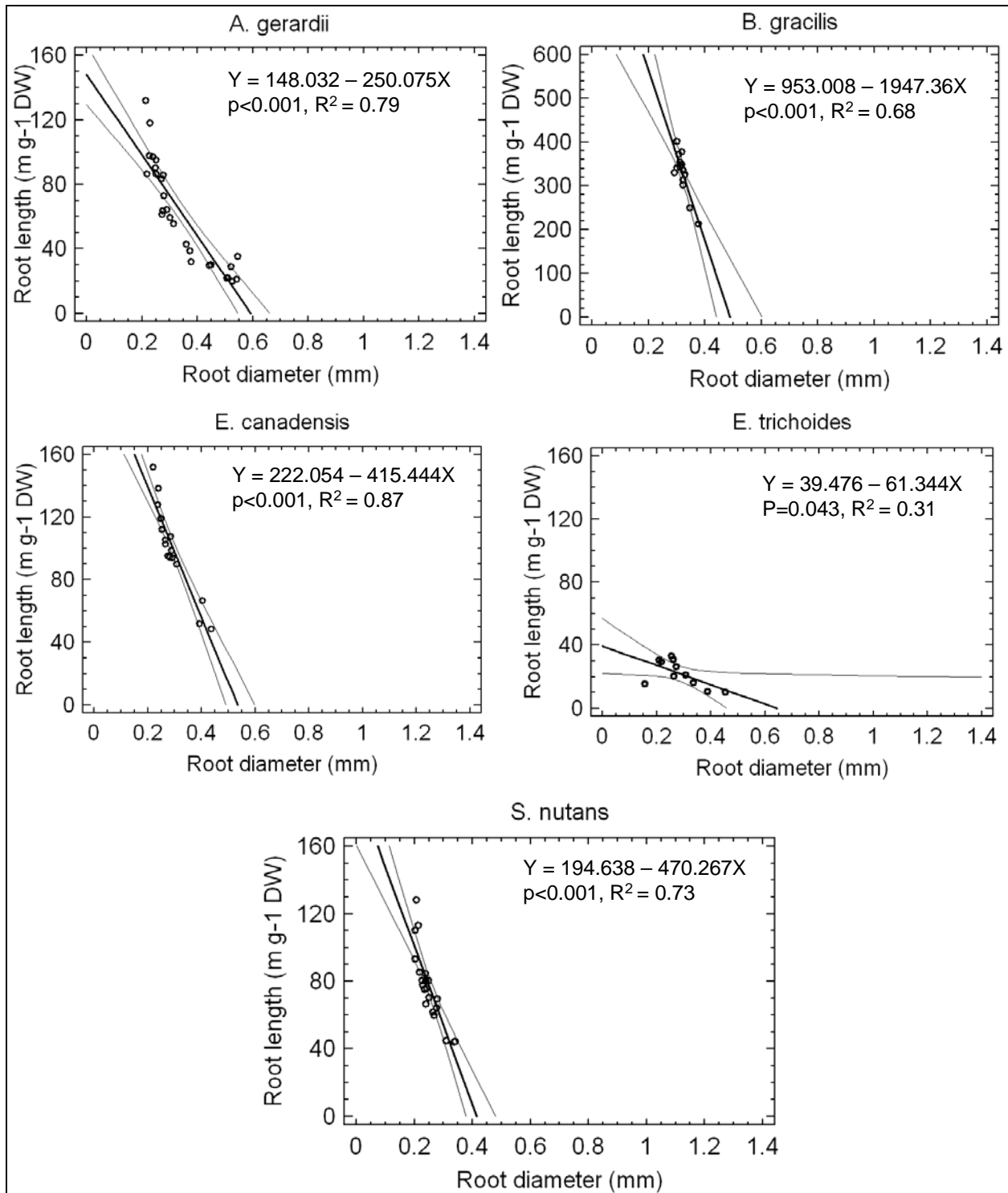


Figure 11. Relationship between root diameter and root length of individual grass species in response to 61 days of exposure to TNT-contaminated soil. Regression lines and 95% confidence limits indicated; Y = plant response, X = target explosives concentration soil mixture.

Specific root surface area ranged from 0.02 m² g⁻¹ DW in *E. trichoides* to 0.32 m² g⁻¹ DW in *B. gracilis* controls (Table 4). The large specific root surface area in *B. gracilis* may explain the elevated sensitivity of this species to TNT due to the far larger contact potential with soil TNT compared to the other grasses.

The evapotranspiration rate in control pots decreased in the order *A. gerardii* > *S. nutans* > *E. canadensis* > *E. trichoides* > *B. gracilis* and ranged from 2.40 L m⁻² d⁻¹ in *A. gerardii* to 1.40 L m⁻² d⁻¹ in *B. gracilis* (Table 4). Evapotranspiration was greater in the pots vegetated with “healthy” grasses (2.09–2.60 L m⁻² d⁻¹; Table 4) than in the other pots (1.34–1.95 L m⁻² d⁻¹; Table 4). Differences in photosynthetic metabolism, potentially causing two times higher water use efficiencies in C₃ than in C₄ species, were apparently less important determinants of evapotranspiration than cultivation conditions (irradiance, watering regime, soil amendment with explosives). The latter can be concluded from the fact that evapotranspirations in *A. gerardii* (C₄), *E. canadensis* (C₃), and *S. nutans* (C₄) controls were similar; while based on their photosynthetic metabolism pathway, it was expected that evapotranspiration in *A. gerardii* and *S. nutans* would be two times lower than in *E. canadensis*. The evapotranspiration rate in the pots with the greatest soil TNT levels in which the grass vegetation grew least or not at all varied over a relatively small range of 1.56 to 1.88 L m⁻² d⁻¹. The latter level can be considered as representative for evapotranspiration of bare soil under typical central Mississippi weather conditions.

Forbs

Plant biomass production of the forbs was significantly affected by TNT concentration ($p < 0.001$), species ($p < 0.001$), and by their interaction ($p < 0.001$; Table 5). The block effects were not statistically significant ($p = 0.507$); therefore, all data were analyzed as if completely randomized. Because the interaction term was significant, the overall TNT exposure effect could not be separated from the species effect. As shown in Table 5 and Figure 6, plant biomass production varied greatly with species, being on the same order of magnitude in *A. retroflexus*, *A. syriaca*, *P. oleracea*, and *S. spinosa*, but significantly greater in *I. lacunosa*. Plant biomass production was significantly affected by TNT exposure in all forbs (Table 6). Production usually decreased with increasing soil TNT concentration up to 100 mg kg⁻¹ soil, and it was completely inhibited in *P. oleracea* at a soil TNT level of 100 mg kg⁻¹ (Table 6, Figure 12).

Table 5. Plant biomass of forbs in response to 55–83 days of exposure to TNT-contaminated soil. Mean values and standard deviations are shown (N=7). Values that are followed by the same letter are not significantly different according to Fisher's least significant difference procedure. ANOVA¹ results are listed.

TNT Exposure Forbs			
Species	Plant Biomass (g DW m ⁻²)		
<i>A. retroflexus</i>	300.08 (224.09) a		
<i>A. syriaca</i>	292.51 (207.86) a		
<i>I. lacunosa</i>	1,476.79 (408.15) c		
<i>P. oleracea</i>	232.66 (219.13) a		
<i>S. spinosa</i>	377.89 (216.36) b		
ANOVA ¹			
Factor	MS	F-ratio	p-value
TNT-exposure	2.13 x 10 ⁶	100.69	<0.001
Species No.	7.76 x 10 ⁶	366.18	<0.001
TNT-exposure x Species No.	49,286	2.32	0.011
¹ ANOVA results of plant biomass data, using target explosives concentration, species, and their interaction as factors (species entered as number in the analysis). Underlining marks a statistically significant effect.			

Table 6. Plant biomass of individual forb species in response to 55–83 days of exposure to TNT-contaminated soil. Mean values and standard deviations are shown (N=7). Values that are followed for the same letter are not significantly different according to Fisher's least significant difference procedure. ANOVA¹ results are listed.

TNT Exposure Forbs					
Factor	Plant Biomass (g DW m ⁻²)				
TNT Exposure	<i>A. retroflexus</i>	<i>A. syriaca</i>	<i>I. lacunosa</i>	<i>P. oleracea</i>	<i>S. spinosa</i>
Control	470.55 (123.82) c	463.77 (165.69) c	1,751.6 (221.8) c	451.47 (40.40) b	574.78 (63.76) b
10 mg kg ⁻¹ TNT	517.43 (86.90) c	460.13 (85.67) c	1,759.7 (207.4) c	435.98 (29.57) b	529.73 (119.40) b
50 mg kg ⁻¹ TNT	174.74 (110.38) b	211.58 (73.06) b	1,425.1 (281.3) b	48.10 (73.61) a	245.83 (128.69) a
100 mg kg ⁻¹ TNT	37.62 (56.65) a	34.58 (45.07) a	970.8 (308.4) a	0 a	161.21 (168.87) a
ANOVA ¹					
Factor	MS	F-ratio	p-value		
TNT-exposure AR	375,422.0	39.23	<0.001		
TNT-exposure AS	304,519.0	28.89	<0.001		
TNT-exposure IL	966,488.0	14.51	<0.001		
TNT-exposure PO	386,639.0	210.03	<0.001		
TNT-exposure SS	294,491.0	18.58	<0.001		
¹ ANOVA results of plant biomass data, using target explosives concentration as factor. Underlining marks a statistically significant effect.					

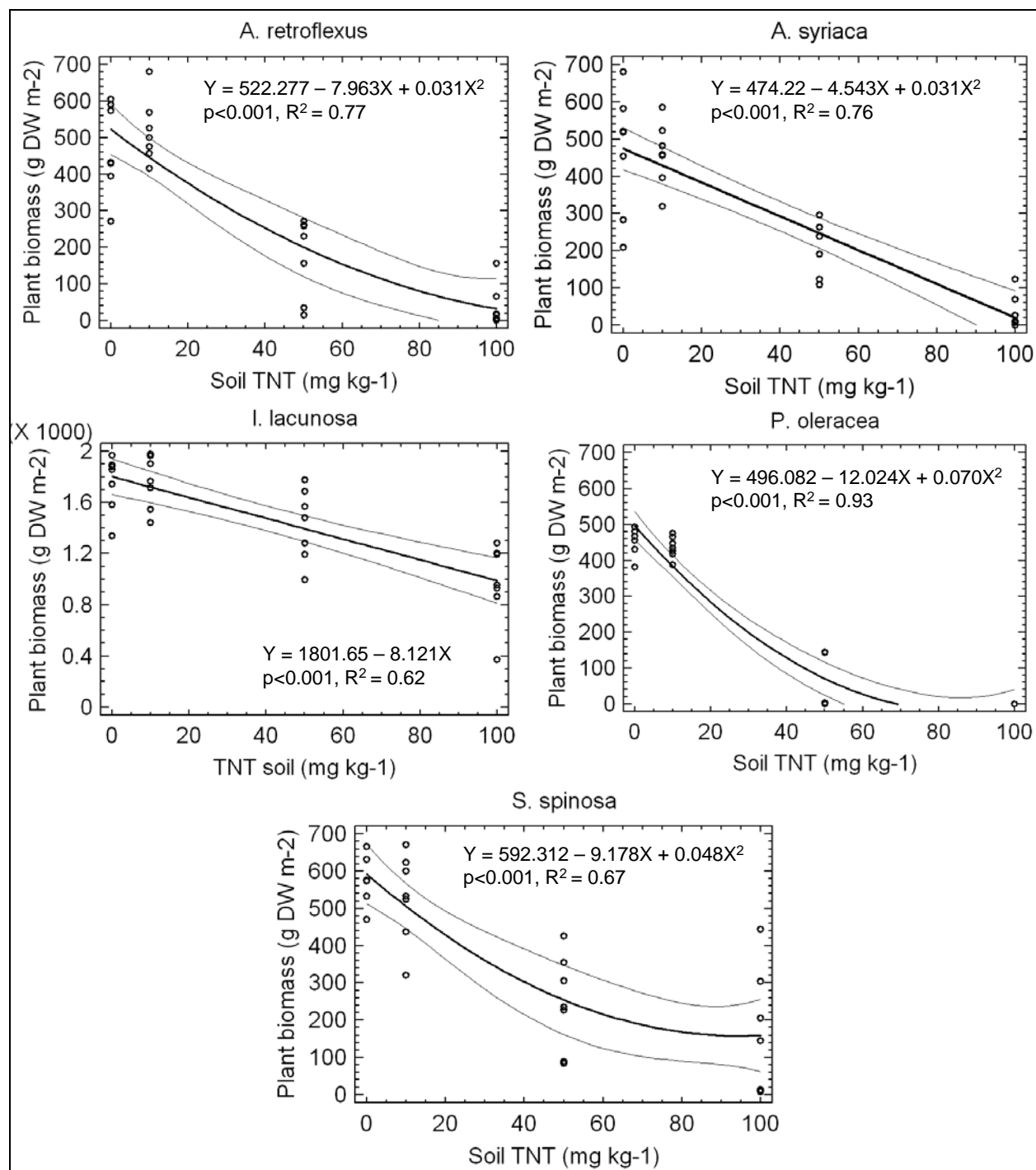


Figure 12. Plant biomass of individual forb species in response to 55–83 days of exposure to TNT-contaminated soil. Regression lines and 95% confidence limits indicated; Y = plant response, X = target explosives concentration soil.

Based on the criterion of having a healthy appearance, the following forb species x treatment combinations were characterized as “healthy”:

- *A. retroflexus*, control, 10 mg, 50 mg TNT kg⁻¹ soil
- *A. syriaca*, control, 10 mg, 50 mg TNT kg⁻¹ soil
- *I. lacunosa*, control, 10 mg, 50, 100 mg TNT kg⁻¹ soil
- *P. oleracea*, control, 10 mg TNT kg⁻¹ soil
- *S. spinosa*, control, 10 mg, 50 mg TNT kg⁻¹ soil

The S:R ratio in the forb controls ranged from 1 in *A. syriaca*, and 3 in *I. lacunosa*, *P. oleracea*, and *S. spinosa*, to 8 in *A. retroflexus* (Table 7; Figure 12). The S:R ratio exhibited a significant relationship with soil TNT level in three forbs, i.e., *I. lacunosa*, *P. oleracea*, and *S. spinosa* (Figure 13). In *I. lacunosa*, it decreased with increasing soil TNT level. In *P. oleracea*, the S:R ratio showed a slight increase upon exposure up to 50 mg TNT kg⁻¹ soil, followed by a decrease, just as in the grass *A. gerardii* (Figure 13). In *S. spinosa*, the S:R ratio increased with increasing soil TNT level (Figure 13).

Root characteristics varied greatly with species. Specific root length in the controls decreased in the order *I. lacunosa* (98 m g⁻¹ DW) > *S. spinosa* (22 m g⁻¹ DW) ≥ *A. syriaca* (21 m g⁻¹ DW) > *A. retroflexus* (14 m g⁻¹ DW) > *P. oleracea* (9 m g⁻¹ DW; Table 7). Root diameter in control plants ranged from 0.52 to 0.95 mm (Table 7). Specific root length of the TNT-exposed plants tended to stay on the same order of magnitude as those of their specific controls, but root length of the scarce *A. retroflexus* plants that tolerated high TNT levels greatly exceeded that of their specific controls, greatly increasing mean values and standard deviations (Table 7; Figure 14). Significant, linear, species-characteristic relationships between root diameter and specific root length, as reported in the grasses (see above), were found only in

- *I. lacunosa*: Root diameter 2.09 mm, specific root length 124 m g⁻¹ DW
- *P. oleracea*: Root diameter 1.54 mm, specific root length 18 m g⁻¹ DW

Specific root surface area ranged from 0.02 m² g⁻¹ DW in *A. retroflexus* and *P. oleracea* to 0.20 m² g⁻¹ DW in *I. lacunosa* controls (Table 7). It increased with increasing TNT exposure level in all forbs (Table 7).

Evapotranspiration was not measured in forbs.

Table 7. Shoot:root ratio and root characteristics of individual forb species in response to 55–83 days of exposure to TNT-contaminated soil. Mean values and standard deviations are shown (N=7). A = absent.

TNT Exposure Forbs				
TNT Exposure	S:R Ratio	Root Characteristics		
		Length (m g ⁻¹ DW)	Diameter (mm)	Surface Area (m ² g ⁻¹ DW)
<i>A. retroflexus</i>				
Control	7.5 (1.7)	13.8 (3.5)	0.52 (0.05)	0.02 (0.01)
10 mg kg ⁻¹ TNT	7.7 (2.2)	9.3 (1.6)	0.63 (0.06)	0.02 (0.00)
50 mg kg ⁻¹ TNT	10.6 (4.5)	55.0 (83.0)	0.93 (0.26)	0.20 (0.31)
100 mg kg ⁻¹ TNT	15.6 (23.5)	87.5 (100.5)	1.08 (0.26)	0.33 (0.38)
<i>A. syriaca</i>				
Control	0.9 (0.3)	20.8 (3.2)	0.95 (0.18)	0.06 (0.01)
10 mg kg ⁻¹ TNT	1.0 (0.2)	21.4 (3.7)	0.84 (0.25)	0.05 (0.01)
50 mg kg ⁻¹ TNT	1.1 (0.4)	23.0 (5.2)	0.73 (0.19)	0.05 (0.02)
100 mg kg ⁻¹ TNT	1.1 (0.8)	45.2 (47.2)	1.05 (0.55)	0.18 (0.23)
<i>I. lacunosa</i>				
Control	3.0 (0.7)	97.9 (20.8)	0.64 (0.09)	0.20 (0.04)
10 mg kg ⁻¹ TNT	3.3 (1.2)	70.4 (6.4)	0.74 (0.15)	0.16 (0.03)
50 mg kg ⁻¹ TNT	2.2 (0.7)	84.2 (8.8)	0.69 (0.15)	0.18 (0.03)
100 mg kg ⁻¹ TNT	1.8 (0.5)	78.4 (17.9)	0.71 (0.07)	0.17 (0.04)
<i>P. oleracea</i>				
Control	3.3 (0.6)	8.7 (5.4)	0.82 (0.33)	0.02 (0.01)
10 mg kg ⁻¹ TNT	4.6 (1.1)	9.9 (2.9)	0.65 (0.07)	0.02 (0.00)
50 mg kg ⁻¹ TNT	2.1 (2.0)	1.6	1.3	0.1
100 mg kg ⁻¹ TNT	A	A	A	A
<i>S. spinosa</i>				
Control	2.9 (0.7)	21.5 (4.2)	0.52 (0.08)	0.03 (0.01)
10 mg kg ⁻¹ TNT	3.2 (1.0)	22.7 (4.0)	0.70 (0.19)	0.05 (0.01)
50 mg kg ⁻¹ TNT	3.2 (1.2)	27.3 (7.7)	0.67 (0.16)	0.06 (0.01)
100 mg kg ⁻¹ TNT	5.5 (4.5)	18.4 (9.9)	0.69 (0.16)	0.05 (0.01)

RDX exposures

Grasses

Plant biomass production of the grasses was significantly affected by RDX concentration ($p < 0.001$), species ($p < 0.001$), and by their interaction ($p = 0.032$; Table 8). The block effects were not statistically significant ($p = 0.083$); therefore, all data were analyzed as if completely randomized.

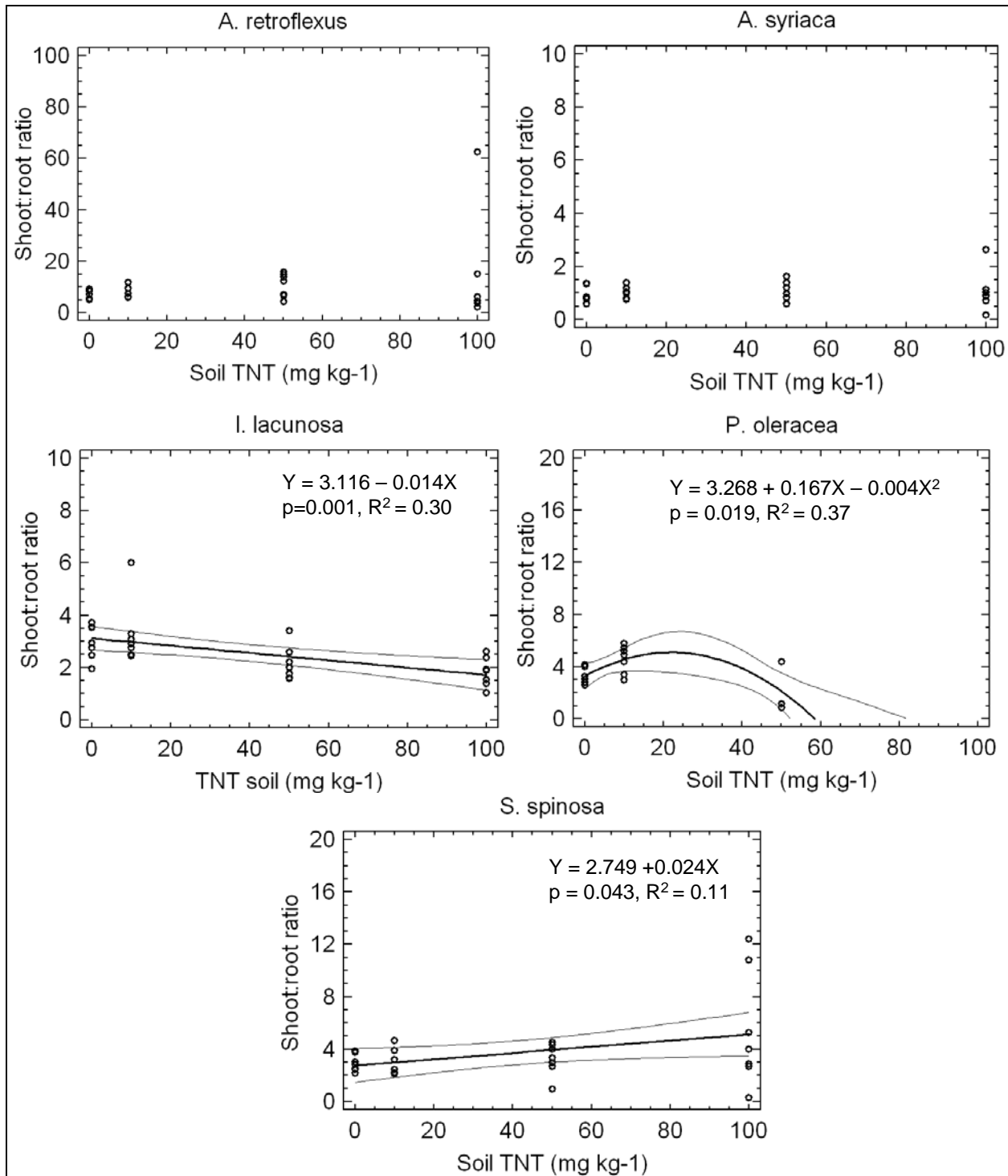


Figure 13. Shoot:root ratio of individual forb species in response to 55–83 days of exposure to TNT-contaminated soil. Regression lines and 95% confidence limits indicated where $p < 0.05$; Y = plant response, X = target explosives concentration soil mixture.

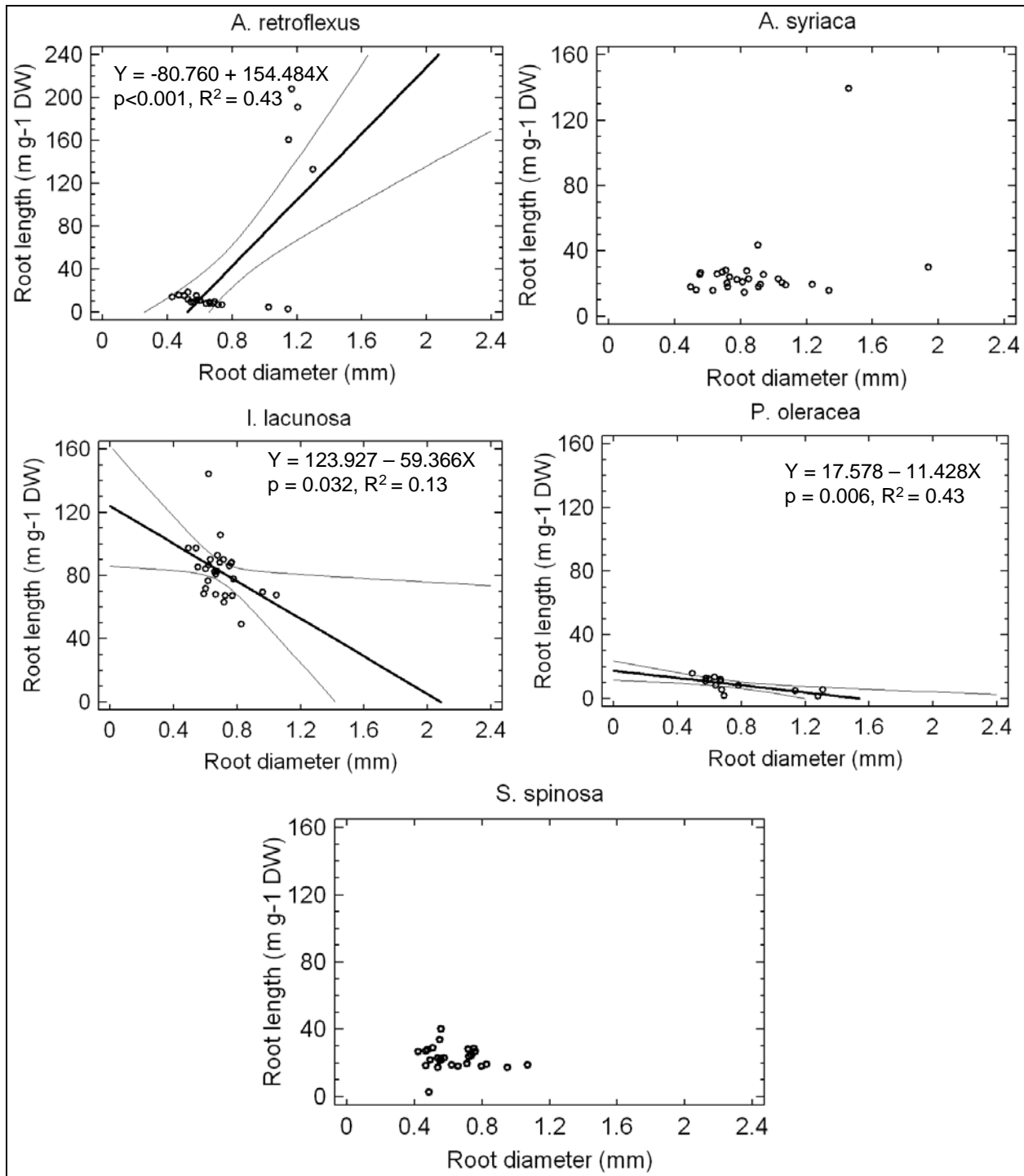


Figure 14. Relationship between root diameter and root length of individual forb species in response to 55–83 days of exposure to TNT-contaminated soil. Regression lines and 95% confidence limits indicated where $p < 0.05$; Y = plant response, X = target explosives concentration soil mixture.

Table 8. Plant biomass of grasses in response to 61 days of exposure to RDX-contaminated soil. Mean values and standard deviations are shown (N=7). Values that are followed by the same letter are not significantly different according to Fisher's least significant difference procedure. ANOVA¹ results are listed.

RDX Exposure Grasses			
Species	Plant Biomass (g DW m ⁻²)		
<i>A. gerardii</i>	331.61 (44.33) d		
<i>B. gracilis</i>	27.49 (8.28) b		
<i>E. canadensis</i>	101.55 (21.08) c		
<i>E. trichoides</i>	5.33 (6.72) a		
<i>S. nutans</i>	397.55 (67.51) e		
ANOVA ¹			
Factor	MS	F-ratio	p-value
RDX-exposure	8,704.5	7.56	<0.001
Species No.	901,518.0	783.34	<0.001
RDX-exposure x Species No.	1,150.9	1.98	0.032
¹ ANOVA results of plant biomass data, using target explosives concentration, species, and their interaction as factors (species entered as number in the analysis). Underlining marks a statistically significant effect.			

Because the interaction term was significant, the overall RDX exposure effect could not be separated from the species effect. As shown in Table 8 and Figure 15, plant biomass production varied greatly with species, being very low in *B. gracilis* and *E. trichoides*, intermediate in *E. canadensis*, and significantly greater in *A. gerardii* and *S. nutans*. Overall germination in *E. trichoides* was very poor, and, therefore, these plants were only analyzed for selected parameters. Plant biomass production was significantly affected by RDX exposure in only one grass species, i.e., *S. nutans* (Table 9). Production usually decreased with increasing RDX concentration up to 1,000 mg kg⁻¹ soil (Table 9, Figure 16). Inhibitions of shoot and root formation by soil RDX level were on the same order of magnitude, and, therefore, no significant trend or relationship was found between soil RDX level and S:R ratio (Table 10; Figure 17). Based on the criterion of having a healthy appearance, all grass species x treatment combinations, except those pertaining to *E. trichoides*, were characterized as “healthy,” i.e., *A. gerardii*, *B. gracilis*, *E. canadensis*, and *S. nutans*.

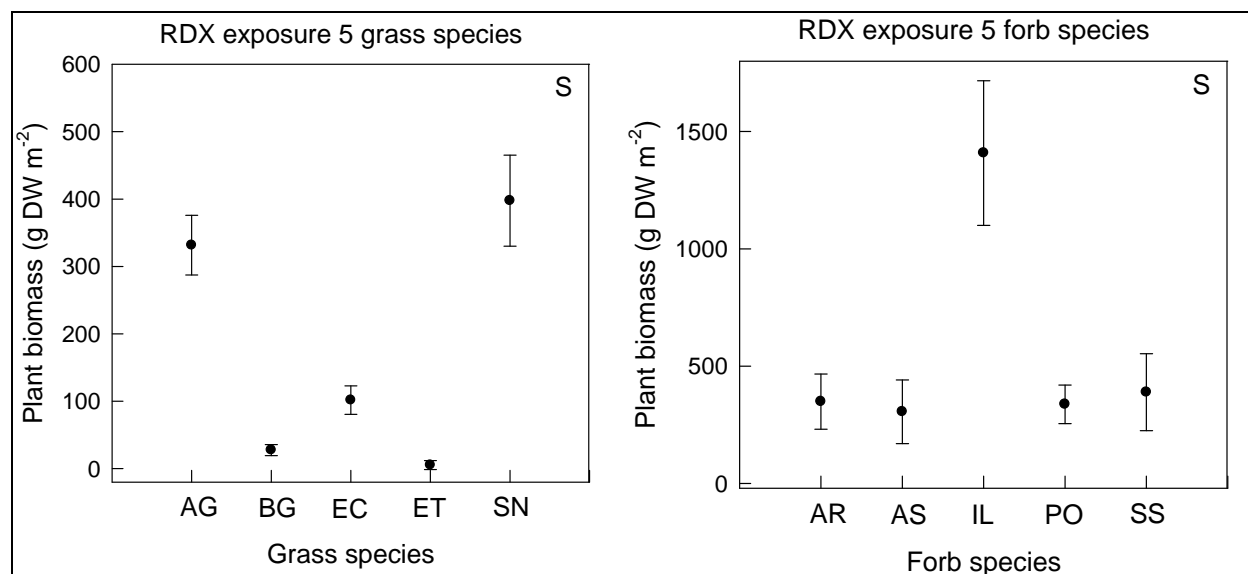


Figure 15. Biomass of plants in response to 61 days of exposure to RDX-contaminated soil. Mean values and standard deviations. Abbreviations: AG - *A. gerardii*; BG - *B. gracilis*; EC - *E. canadensis*; ET - *E. trichoides*; SN - *S. nutans*; AR - *A. retroflexus*; AS - *A. syriaca*; IL - *I. lacunosa*; PO - *P. oleracea*; SS - *S. spinosa*; S is statistically significant.

Table 9. Plant biomass of individual grass species in response to 61 days of exposure to RDX-contaminated soil. Mean values and standard deviations are shown (N=7). Values that are followed by the same letter are not significantly different according to Fisher's least significant difference procedure. ANOVA¹ results are listed.

RDX Exposure Grasses					
Factor	Plant Biomass (g DW m ⁻²)				
RDX Exposure	<i>A. gerardii</i>	<i>B. gracilis</i>	<i>E. canadensis</i>	<i>E. trichoides</i>	<i>S. nutans</i>
Control	353.63 (39.58) c	31.18 (9.55) a	116.78 (16.00) b	9.93 (7.07) a	454.65 (63.51) b
100 mg kg ⁻¹ RDX	337.76 (55.95) ab	27.66 (8.39) a	102.91 (26.85) ab	6.16 (8.75) a	375.67 (63.21) ab
500 mg kg ⁻¹ RDX	330.43 (41.04) ab	24.62 (7.97) a	99.20 (13.31) ab	3.10 (5.09) a	408.57 (55.99) a
1,000 mg kg ⁻¹ RDX	304.61 (31.64) a	26.49 (7.55) a	87.33 (18.21) a	2.80 (4.07) a	351.32 (48.49) a
ANOVA ¹					
Factor	MS	F-ratio	p-value		
RDX-exposure AG	2,923.8	1.58	0.219		
RDX-exposure BG	53.3	0.76	0.530		
RDX-exposure EC	1,030.2	2.77	0.063		
RDX-exposure ET	70.4	1.68	0.199		
RDX-exposure SN	13,993.7	4.14	<u>0.017</u>		
¹ ANOVA results of plant biomass data, using target explosives concentration as factor. Underlining marks a statistically significant effect.					

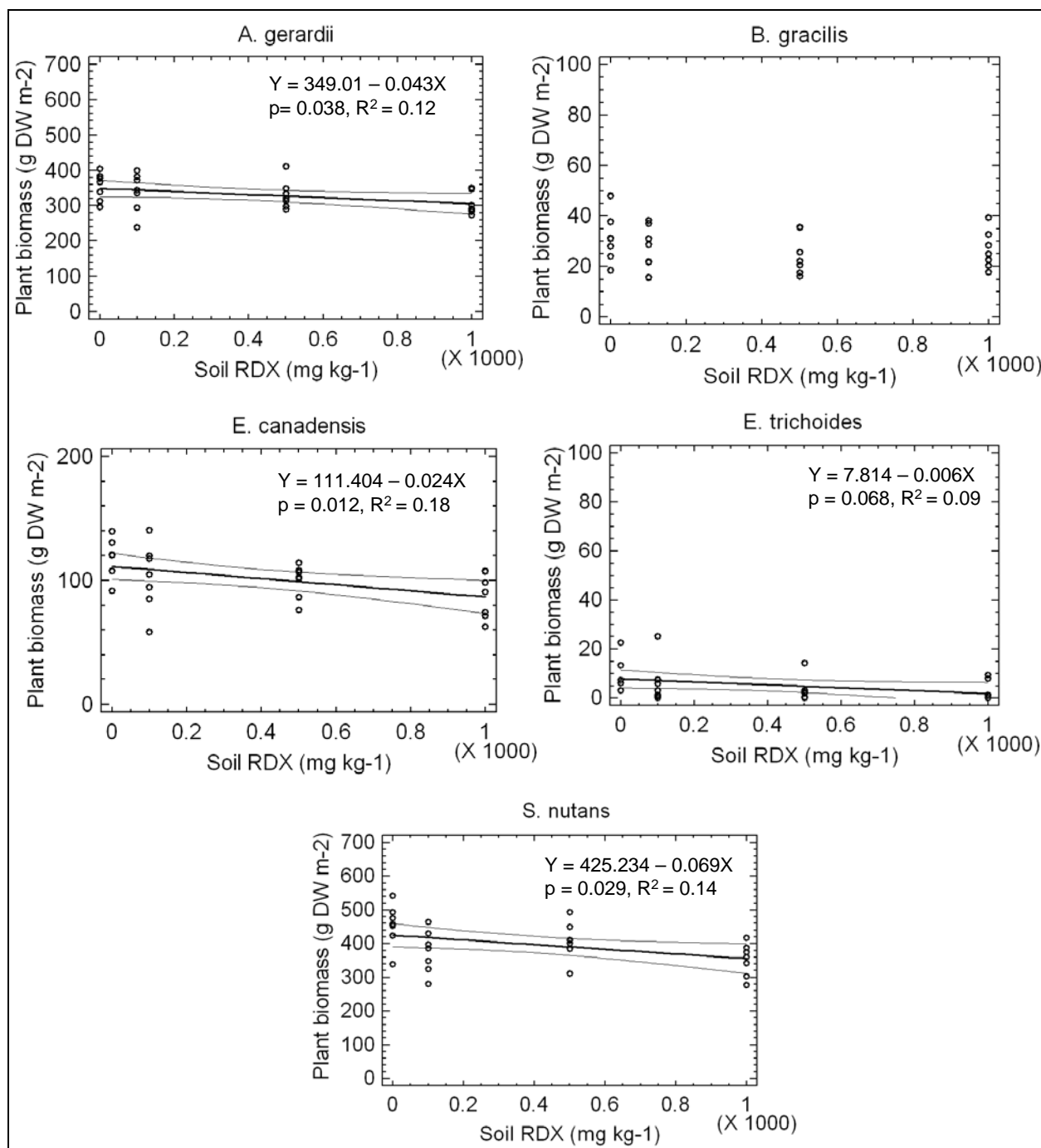


Figure 16. Plant biomass of individual grass species in response to 61 days of exposure to RDX-contaminated soil. Regression lines and 95% confidence limits indicated where $p < 0.05$; Y = plant response, X = target explosives concentration soil mixture.

Table 10. Shoot:root ratio, root characteristics, and evapotranspiration rates of individual grass species in response to 61 days of exposure to RDX-contaminated soil. Mean values and standard deviations are shown (N=7).

RDX Exposure Grasses					
RDX Exposure	S:R Ratio	Root Characteristics			Evapotranspiration (L m ⁻² d ⁻¹)
		Length (m g ⁻¹ DW)	Diameter (mm)	Surface Area (m ² g ⁻¹ DW)	
<i>A. gerardii</i>					
Control	1.8 (0.3)	99.8 (19.6)	0.24 (0.02)	0.07 (0.01)	2.40
100 mg kg ⁻¹ RDX	1.8 (0.4)	67.0 (12.9)	0.27 (0.02)	0.06 (0.01)	2.43
500 mg kg ⁻¹ RDX	2.4 (0.6)	59.3 (9.0)	0.28 (0.03)	0.05 (0.00)	2.43
1,000 mg kg ⁻¹ RDX	2.2 (0.5)	80.5 (36.7)	0.27 (0.06)	0.06 (0.02)	2.44
<i>B. gracilis</i>					
Control	5.5 (1.8)	325.8 (40.0)	0.32 (0.02)	0.32 (0.03)	1.40
100 mg kg ⁻¹ RDX	5.3 (1.4)	418.4 (93.6)	0.29 (0.03)	0.38 (0.05)	1.18
500 mg kg ⁻¹ RDX	4.4 (1.3)	388.6 (64.9)	0.30 (0.02)	0.37 (0.04)	1.27
1,000 mg kg ⁻¹ RDX	5.5 (1.0)	458.9 (39.7)	0.28 (0.02)	0.41 (0.02)	1.19
<i>E. canadensis</i>					
Control	1.7 (0.2)	112.1 (14.9)	0.26 (0.02)	0.09 (0.01)	2.14
100 mg kg ⁻¹ RDX	1.8 (0.3)	108.4 (24.1)	0.27 (0.03)	0.09 (0.01)	2.12
500 mg kg ⁻¹ RDX	1.9 (0.4)	88.5 (26.0)	0.30 (0.05)	0.08 (0.01)	2.18
1,000 mg kg ⁻¹ RDX	1.8 (0.2)	87.0 (21.6)	0.32 (0.05)	0.08 (0.01)	1.96
<i>E. trichoides</i>					
Control	3.9 (2.8)	29.2 (2.0)	0.24 (0.03)	0.02 (0.00)	1.69
100 mg kg ⁻¹ RDX	4.3 (2.9)	25.0 (6.8)	0.21 (0.07)	0.02 (0.01)	1.70
500 mg kg ⁻¹ RDX	3.6 (1.7)	42.1 (42.9)	0.20 (0.13)	0.03 (0.03)	1.69
1,000 mg kg ⁻¹ RDX	1.7 (0.2)	33.2 (2.5)	0.27 (0.01)	0.03 (0.00)	1.66
<i>S. nutans</i>					
Control	2.6 (0.6)	92.7 (24.6)	0.23 (0.02)	0.09 (0.01)	2.23
100 mg kg ⁻¹ RDX	2.6 (1.2)	70.7 (32.8)	0.25 (0.04)	0.06 (0.01)	2.09
500 mg kg ⁻¹ RDX	3.2 (1.0)	63.0 (17.1)	0.27 (0.04)	0.05 (0.01)	2.07
1,000 mg kg ⁻¹ RDX	2.7 (0.5)	79.3 (12.4)	0.25 (0.02)	0.06 (0.01)	2.03

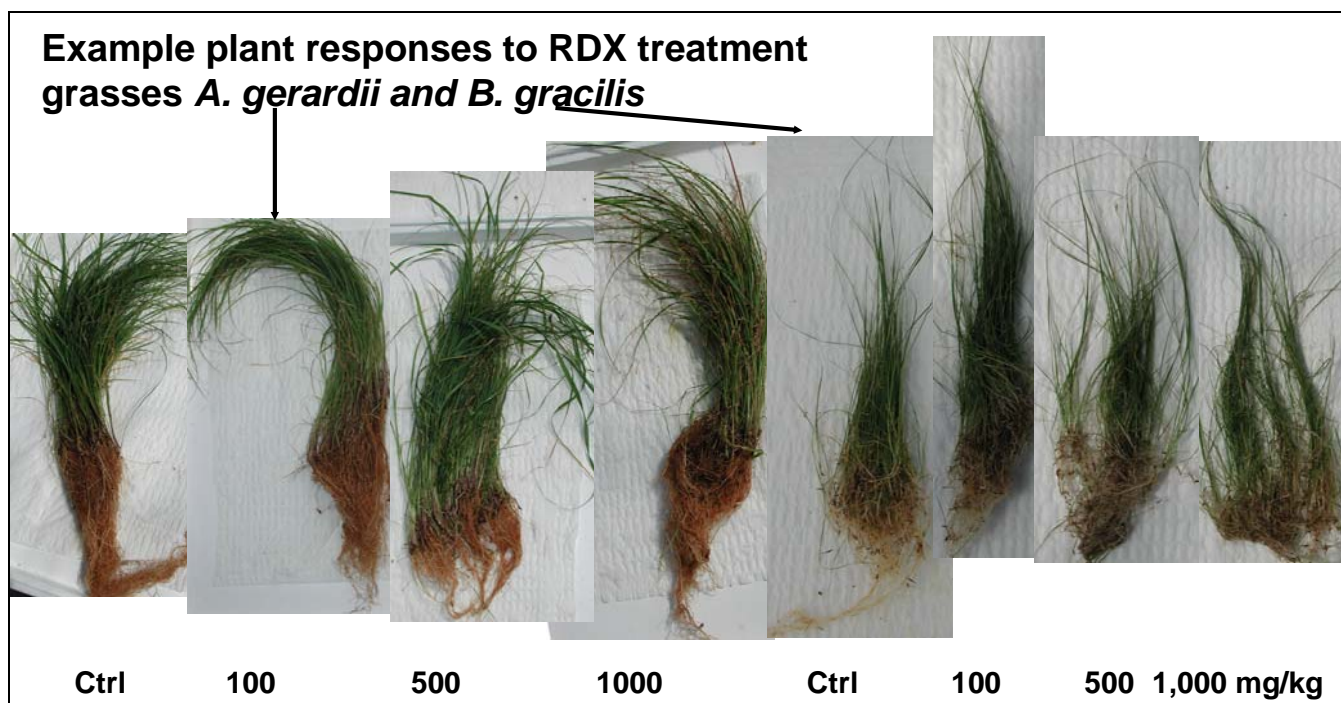


Figure 17. Typical response of two grass species to 61 days of exposure to RDX-contaminated soil illustrating that root systems were not significantly reduced.

Specific root length decreased with increasing soil RDX level in three grass species, i.e., *A. gerardii*, *E. canadensis*, *S. nutans*, but increased in the two other grasses, *B. gracilis* and *E. trichoides* (Table 10). Root diameter increased with increasing soil RDX in the same grass species in which root length decreased, whereas root diameter did not show a clear trend in *B. gracilis* and *E. trichoides* (Table 10). Significant, linear, species-characteristic relationships between root diameter and specific root length were also apparent in this case in four of the five grass species when exposed to soil-based RDX, indicating critical combinations of maximum root diameter and maximum specific root length for grasses upon exposure to RDX. No such relationship was established for *E. trichoides* because of insufficient data availability (Figure 18).

The following critical combinations were calculated using the regression equations:

- Root diameter 0.39 mm, specific root length 240 m g⁻¹ DW in *A. gerardii*
- Root diameter 0.45 mm, specific root length 1,172 m g⁻¹ DW in *B. gracilis*

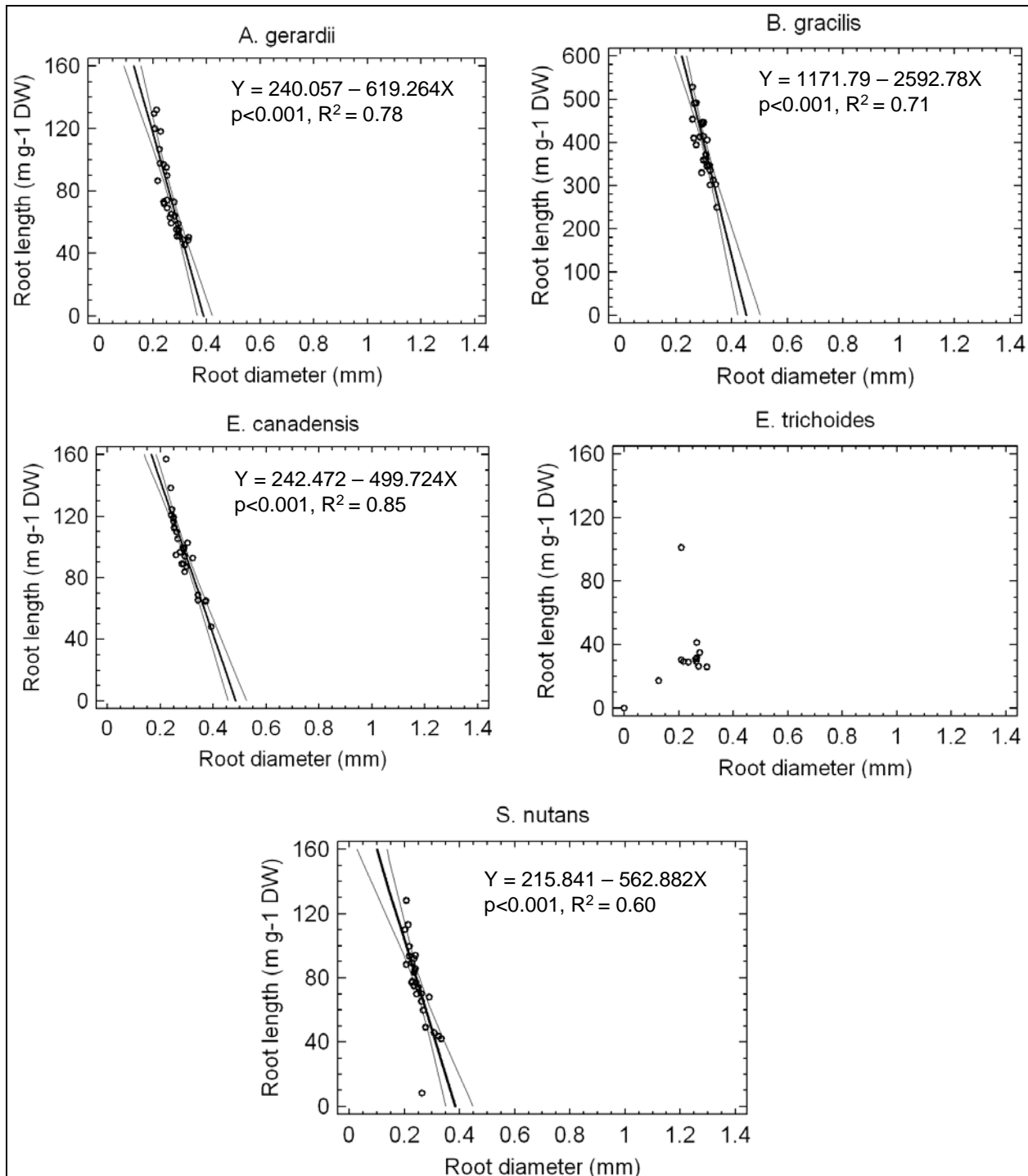


Figure 18. Relationship between root diameter and root length of individual grass species in response to 61 days of exposure to RDX-contaminated soil. Regression lines and 95% confidence limits indicated where $p < 0.05$; Y = plant response, X = target explosives concentration soil mixture.

- Root diameter 0.48 mm, specific root length 242 m g⁻¹ DW in *E. canadensis*
- Root diameter 0.38 mm, specific root length 215 m g⁻¹ DW in *S. nutans*

Specific root surface area of RDX-exposed plants remained in the same order of magnitude as those of the specific controls in the three species *A. gerardii*, *E. canadensis*, and *E. trichoides*. It increased with soil RDX level in *B. gracilis* and decreased in *S. nutans* (Table 10).

Evapotranspiration was greater in the pots vegetated with “healthy” grasses (1.96–2.44 L m⁻² d⁻¹; Table 10) than in the other pots (1.19–1.70 L m⁻² d⁻¹), as with the TNT-exposed grasses. The evapotranspiration rate in the pots in which the grass vegetation grew least or not at all varied over a relatively small range of 1.19 to 1.70 L m⁻² d⁻¹. The latter level is close to the level of 1.56–1.88 L m⁻² d⁻¹ derived from the TNT-exposed grass-vegetated pots, and expands the range of evapotranspiration considered as representative for evapotranspiration of bare soil under typical central Mississippi weather conditions to 1.19–1.88 L m⁻² d⁻¹.

Forbs

Plant biomass production of the forbs was significantly affected by RDX concentration ($p < 0.001$), species ($p < 0.001$), and by their interaction ($p < 0.001$; Table 11). The block effects were not statistically significant ($p = 0.353$); therefore, all data were analyzed as if completely randomized. Because the interaction term was significant, the overall RDX exposure effect could not be separated from the species effect, being in the same order of magnitude in *A. retroflexus*, *A. syriaca*, *P. oleracea*, and *S. spinosa*, and significantly greater in *I. lacunosa*. Plant biomass production was significantly affected by RDX exposure in all forbs (Table 12). Plant biomass usually decreased with increasing soil RDX concentration up to 1,000 mg kg⁻¹ soil (Table 12, Figure 19).

Inhibitions of shoot and root production by soil RDX level were on the same order of magnitude, and, therefore, no significant trend or relationship was found between soil RDX level and S:R ratio (Table 13).

Table 11. Plant biomass of forbs in response to 55–83 days exposure to RDX-contaminated soil. Mean values and standard deviations are shown (N=7). Values that are followed by the same letter are not significantly different according to Fisher's least significant difference procedure. ANOVA¹ results are listed.

RDX Exposure Forbs			
Species	Plant Biomass (g DW m ⁻²)		
<i>A. retroflexus</i>	348.74 (117.90) ab		
<i>A. syriaca</i>	305.78 (135.53) a		
<i>I. lacunosa</i>	1,408.45 (308.16) d		
<i>P. oleracea</i>	337.29 (82.07) ab		
<i>S. spinosa</i>	389.04 (164.46) c		
ANOVA ¹			
Factor	MS	F-ratio	p-value
RDX-exposure	668,981.0	53.64	<0.001
Species No.	6.32 x 10 ⁶	507.48	<0.001
RDX-exposure x Species No.	12,472.8	5.46	<0.001
¹ ANOVA results of plant biomass data, using target explosives concentration, species, and their interaction as factors (species entered as number in the analysis). Underlining marks a statistically significant effect.			

Table 12. Plant biomass of individual forb species in response to 55–83 days of exposure to RDX-contaminated soil. Mean values and standard deviations are shown (N=7). Values that are followed for the same letter are not significantly different according to Fisher's least significant difference procedure. ANOVA¹ results are listed.

RDX Exposure Forbs					
Factor	Plant Biomass (g DW m ⁻²)				
	<i>A. retroflexus</i>	<i>A. syriaca</i>	<i>I. lacunosa</i>	<i>P. oleracea</i>	<i>S. spinosa</i>
Control	470.55 (123.82) b	463.77 (165.69) c	1,751.58 (221.77) c	451.47 (40.40) b	574.78 (63.76) c
100 mg kg ⁻¹ RDX	363.81 (90.22) a	310.91 (71.91) b	1,552.38 (141.07) b	303.26 (67.61) a	413.50 (68.85) b
500 mg kg ⁻¹ RDX	264.70 (74.81) a	243.91 (62.15) ab	1,079.11 (167.59) a	287.65 (39.78) a	277.17 (84.06) ab
1,000 mg kg ⁻¹ RDX	295.90 (66.37) a	204.54 (24.40) a	1,250.73 (114.34) a	323.10 (64.63) a	290.72 (197.52) a
ANOVA ¹					
Factor	MS	F-ratio	p-value		
RDX-exposure AR	58,148.5	6.95	<u>0.002</u>		
RDX-exposure AS	91,153.5	9.83	<0.001		
RDX-exposure IL	634,179.0	23.01	<0.001		
RDX-exposure PO	34,993.7	11.47	<0.001		
RDX-exposure SS	133,653.0	9.74	<0.001		
¹ ANOVA results of plant biomass data, using target explosives concentration as factor. Underlining marks a statistically significant effect.					

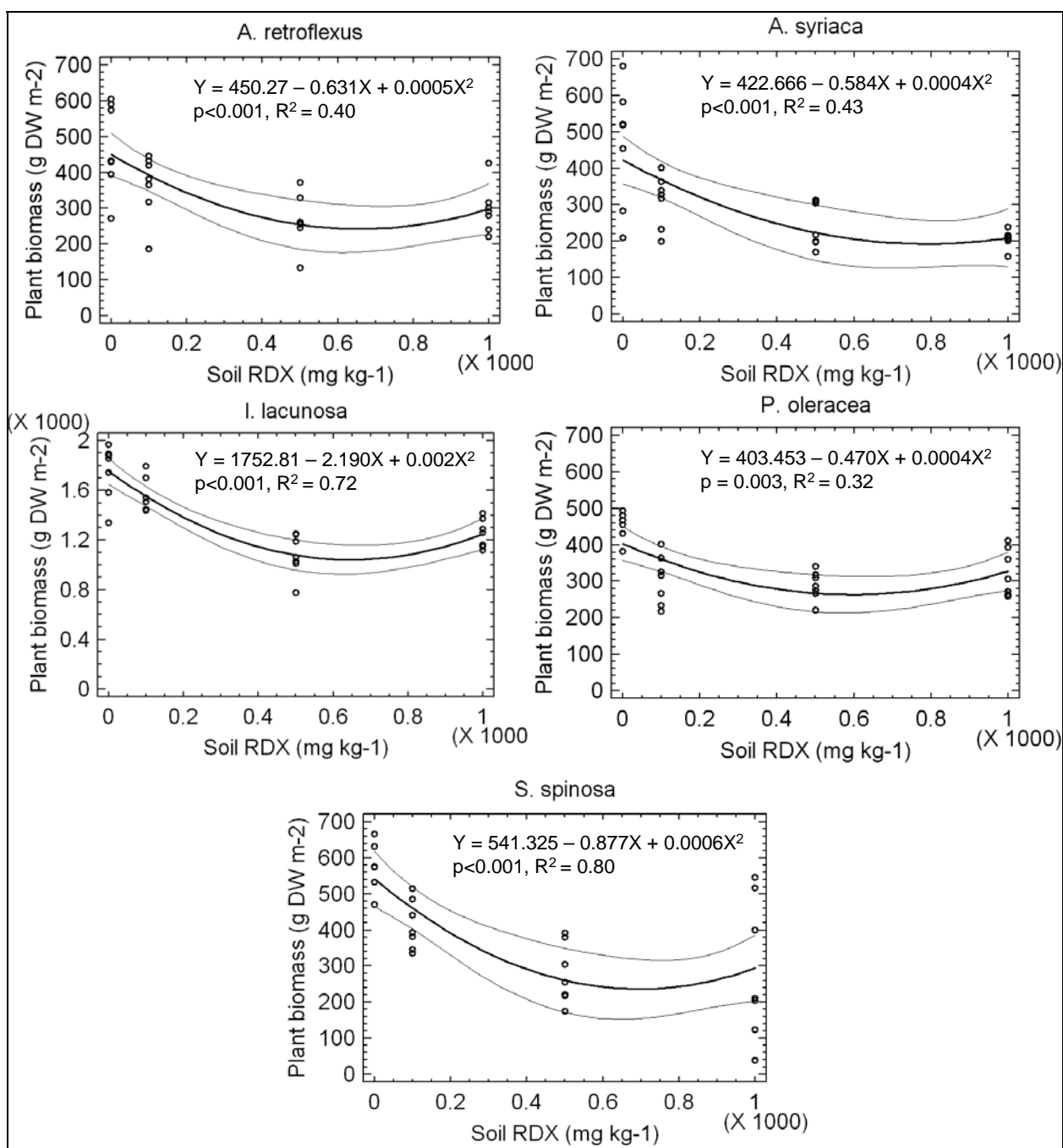


Figure 19. Plant biomass of individual forb species in response to 55–83 days of exposure to RDX-contaminated soil. Regression lines and 95% confidence limits indicated; Y = plant response, X = target explosives concentration soil mixture.

Table 13. Shoot:root ratio and root characteristics of individual forb species in response to 55–83 days of exposure to RDX-contaminated soil. Mean values and standard deviations are shown (N=7).

RDX Exposure Forbs				
RDX Exposure	S:R Ratio	Root Characteristics		
		Length (m g ⁻¹ DW)	Diameter (mm)	Surface Area (m ² g ⁻¹ DW)
<i>A. retroflexus</i>				
Control	7.5 (1.7)	13.8 (3.5)	0.52 (0.05)	0.02 (0.01)
100 mg kg ⁻¹ RDX	3.9 (0.8)	10.0 (3.6)	0.64 (0.11)	0.02 (0.00)
500 mg kg ⁻¹ RDX	3.5 (1.3)	8.9 (1.6)	0.65 (0.07)	0.02 (0.00)
1,000 mg kg ⁻¹ RDX	3.9 (1.5)	10.0 (3.0)	0.70 (0.24)	0.02 (0.00)
<i>A. syriaca</i>				
Control	0.9 (0.3)	20.8 (3.2)	0.95 (0.18)	0.06 (0.01)
100 mg kg ⁻¹ RDX	1.6 (0.3)	24.7 (3.9)	0.82 (0.16)	0.06 (0.02)
500 mg kg ⁻¹ RDX	1.8 (0.5)	24.9 (3.0)	0.81 (0.14)	0.06 (0.01)
1,000 mg kg ⁻¹ RDX	2.3 (0.5)	19.1 (3.7)	0.96 (0.65)	0.06 (0.03)
<i>I. lacunosa</i>				
Control	3.0 (0.7)	97.9 (20.8)	0.64 (0.09)	0.20 (0.04)
100 mg kg ⁻¹ RDX	2.6 (0.4)	104.2 (18.3)	0.64 (0.06)	0.21 (0.04)
500 mg kg ⁻¹ RDX	4.0 (1.0)	80.5 (10.8)	0.67 (0.14)	0.17 (0.03)
1,000 mg kg ⁻¹ RDX	3.7 (0.8)	73.6 (16.4)	0.80 (0.11)	0.18 (0.03)
<i>P. oleracea</i>				
Control	3.3 (0.6)	8.7 (5.4)	0.82 (0.33)	0.02 (0.01)
100 mg kg ⁻¹ RDX	5.0 (1.6)	10.9 (3.1)	0.63 (0.13)	0.02 (0.00)
500 mg kg ⁻¹ RDX	3.3 (0.8)	7.7 (1.6)	0.82 (0.17)	0.02 (0.00)
1,000 mg kg ⁻¹ RDX	4.4 (3.4)	7.6 (1.0)	0.77 (0.07)	0.02 (0.00)
<i>S. spinosa</i>				
Control	2.9 (0.7)	21.5 (4.2)	0.52 (0.08)	0.03 (0.01)
100 mg kg ⁻¹ RDX	3.0 (0.8)	19.5 (3.1)	0.67 (0.15)	0.04 (0.00)
500 mg kg ⁻¹ RDX	2.5 (1.4)	19.4 (4.0)	0.88 (0.24)	0.05 (0.02)
1,000 mg kg ⁻¹ RDX	2.7 (1.2)	17.9 (6.8)	0.87 (0.22)	0.05 (0.01)

Specific root length decreased with increasing soil RDX level in the four forb species (*A. retroflexus*, *I. lacunosa*, *P. oleracea*, and *S. spinosa*), but increased and subsequently decreased in *A. syriaca* (Table 13). Root diameter increased with increasing soil RDX in the same forb species in which root length decreased, whereas root diameter did not show a clear trend in *A. syriaca* (Table 13). Significant, linear, species-characteristic relationships between root diameter and specific root length were found in three forb species when exposed to soil-based RDX (Figure 20). No such relationships were established in *A. syriaca* and *I. lacunosa*. The following critical combinations were calculated using the regression equations:

- Root diameter 1.33 mm, specific root length 20 m g⁻¹ DW in *A. retroflexus*
- Root diameter 1.55 mm, specific root length 17 m g⁻¹ DW in *P. oleracea*
- Root diameter 2.58 mm, specific root length 27 m g⁻¹ DW in *S. spinosa*

The specific root surface areas of RDX-exposed forbs were similar to those of controls and ranged from 0.02 m² g⁻¹ DW in *A. retroflexus* and *P. oleracea* to 0.21 m² g⁻¹ DW in *I. lacunosa* (Table 13).

Evapotranspiration was not measured in forbs.

Energetics mass balances of soil-plant systems

TNT exposures

Grasses

The initial extractable TNT concentrations in the amended soils used for the grass tests were less than the target concentrations and ranged from 2.38 mg kg⁻¹ soil DW (10-mg kg⁻¹ target concentration) to 38.06 mg kg⁻¹ soil (100-mg kg⁻¹ target concentration; Table 14).

The final concentrations of TNT and TNT-equivalents (derived from the measured concentrations of the TNT metabolites 2-ADNT and 4-ADNT by conversion on a molar basis) were below detection in the 10-mg kg⁻¹ soils of the grass tests, and low in the soils with higher target TNT levels (Table 15). TNT loss, expressed as percentage of initial, ranged from 79.0% to 100%, and was greatest in the 10-mg kg⁻¹ soils and least in the 100-mg kg⁻¹ soils (Table 15). Total TNT loss, expressed in milligrams TNT per pot, ranged from 1.83 to 24.26 mg (Table 14, Table 15).

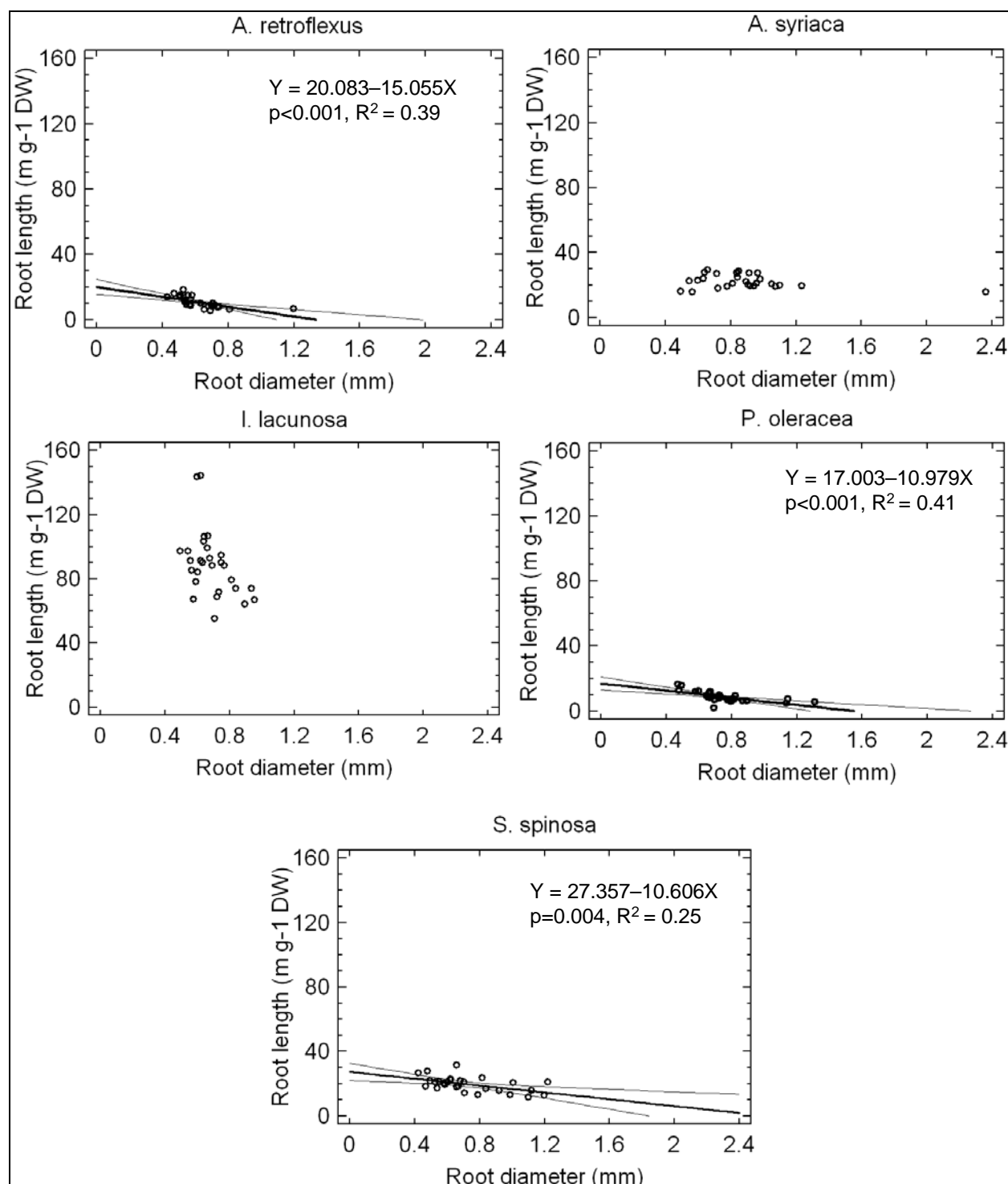


Figure 20. Relationship between root length and root diameter of individual forb species in response to 55–83 days of exposure to RDX-contaminated soil. Regression lines and 95% confidence limits indicated where $p < 0.05$; Y = plant response, X = target explosives concentration soil mixture.

Table 14. Extractable explosives in the amended soil mixtures prior to the tests; concentrations and contents per pot. Mean values and standard deviations are shown (N=3).

Soil Mixture	Initial TNT		Initial RDX		Initial HMX
	(mg kg ⁻¹)	(mg pot ⁻¹)	(mg kg ⁻¹)	(mg pot ⁻¹)	(mg kg ⁻¹)
Grass Tests					
10 mg kg ⁻¹ TNT	2.38 ± 0.30	1.83			
50 mg kg ⁻¹ TNT	18.25 ± 1.25	14.02			
100 mg kg ⁻¹ TNT	38.06 ± 0.64	29.23			
Forb Tests					
10 mg kg ⁻¹ TNT	0.83 ± 0.15	0.64			
50 mg kg ⁻¹ TNT	8.12 ± 0.53	6.24			
100 mg kg ⁻¹ TNT	56.80 ± 2.17	43.62			
Grass Tests					
100 mg kg ⁻¹ RDX			5.09 ± 0.62	3.91	0.72 ± 0.09
500 mg kg ⁻¹ RDX			47.38 ± 5.79	36.39	3.80 ± 1.26
1,000 mg kg ⁻¹ RDX			778.47 ± 100.76	597.50	60.40 ± 5.73
Forb Tests					
100 mg kg ⁻¹ RDX			119.00 ± 5.34	91.39	15.31 ± 1.74
500 mg kg ⁻¹ RDX			346.73 ± 1.61	266.28	38.91 ± 1.54
1,000 mg kg ⁻¹ RDX			1,069.07 ± 20.92	821.05	122.17 ± 6.78
Note: Initial soil weight: 768 g DW pot ⁻¹ .					

The greatest absolute TNT loss of 24.26 mg occurred in pots amended to the 100-mg kg⁻¹ target level, which were vegetated by *S. nutans*. Scaling up TNT loss per pot surface area to TNT loss per hectare, by multiplication with a factor of 76×10^4 , would yield a TNT loss of 18.4 kg ha⁻¹.

The final concentrations of TNT and TNT-equivalents accumulated in grasses were below detection in all species when exposed to the 10-mg kg⁻¹ target TNT concentration, and in two of the five species (*A. gerardii* and *S. nutans*) when exposed to the 50-mg kg⁻¹ target TNT concentration (Table 16). One grass species only, i.e., *A. gerardii*, showed accumulation of TNT + TNT-equivalents in both shoots and roots of 90.7 and 819.8 mg kg⁻¹, respectively, when exposed to the greatest 100-mg kg⁻¹ target TNT concentration. Three other grasses, i.e., *B. gracilis*, *E. canadensis*, and *S. nutans*, showed accumulation to far greater levels (up to 8,765 mg kg⁻¹) in their shoots, but these plants lacked measurable root systems and were, therefore, not viable. The greatest absolute TNT + TNT-equivalent accumulation by viable plants occurred in *A. gerardii* exposed to 100 mg TNT kg⁻¹, amounting to 0.22 mg per pot (0.2 kg ha⁻¹; Table 16).

Table 15. Extractable TNT and TNT-equivalent concentrations of the remediated soil mixtures at the end of the tests, and calculated loss relative to initial. Mean values and standard deviations are shown (N=3).

TNT Exposure	Final Extractable Explosives Concentration Soil (mg kg ⁻¹ DW)		TNT Loss (% initial extractable TNT)
	TNT	TNT + TNT-Equivalents ^a	
Grass Tests			
<i>A. gerardii</i>			
10 mg kg ⁻¹ TNT	<0.168	BD	100
50 mg kg ⁻¹ TNT	1.94 ± 0.67	2.63 ± 1.16	89.4 ± 3.7
100 mg kg ⁻¹ TNT	8.00 ± 1.79	10.54 ± 2.24	79.0 ± 4.7
<i>B. gracilis</i>			
10 mg kg ⁻¹ TNT	<0.168	BD	100
50 mg kg ⁻¹ TNT	3.18 ± 0.39	4.05 ± 1.08	82.6 ± 2.1
100 mg kg ⁻¹ TNT	8.02 ± 0.86	10.02 ± 0.84	78.9 ± 2.3
<i>E. canadensis</i>			
10 mg kg ⁻¹ TNT	<0.168	BD	100
50 mg kg ⁻¹ TNT	2.26 ± 1.06	3.40 ± 1.37	87.6 ± 5.8
100 mg kg ⁻¹ TNT	7.92 ± 0.21	10.05 ± 0.86	79.2 ± 0.5
<i>S. nutans</i>			
10 mg kg ⁻¹ TNT	<0.168	BD	100
50 mg kg ⁻¹ TNT	0.85 ± 0.07	0.90 ± 0.06	95.3 ± 0.3
100 mg kg ⁻¹ TNT	6.46 ± 1.39	8.30 ± 1.52	83.0 ± 3.6
Forb Tests			
<i>A. retroflexus</i>			
10 mg kg ⁻¹ TNT	<0.168	BD	100
50 mg kg ⁻¹ TNT	0.50 ± 0.13	0.91 ± 0.17	88.8 ± 2.2
100 mg kg ⁻¹ TNT	0.57	1.16	98.0
<i>A. syriaca</i>			
10 mg kg ⁻¹ TNT	<0.168	BD	100
50 mg kg ⁻¹ TNT	<0.168	0.24 ± 0.21	99.6 ± 0.4
100 mg kg ⁻¹ TNT	<0.168	0.24 ± 0.21	34.6 ± 2.9
<i>I. lacunosa</i>			
10 mg kg ⁻¹ TNT	<0.168	BD	100
50 mg kg ⁻¹ TNT	<0.168	BD	100
100 mg kg ⁻¹ TNT	<0.168	BD	100
<i>P. oleracea</i>			
10 mg kg ⁻¹ TNT	<0.168	0.09 ± 0.15	86.4 ± 23.6
50 mg kg ⁻¹ TNT	0.34	0.81	90.0
100 mg kg ⁻¹ TNT	A	A	A
<i>S. spinosa</i>			
10 mg kg ⁻¹ TNT	0.08 ± 0.14	0.18 ± 0.30	72.3 ± 48.0
50 mg kg ⁻¹ TNT	0.33 ± 0.17	0.78 ± 0.17	90.3 ± 2.1
100 mg kg ⁻¹ TNT	0.26 ± 0.22	0.65 ± 0.56	98.8 ± 1.0
Note: <i>E. trichoides</i> soils were not analyzed because plants failed to grow; BD = below detection; A = absent (soils not analyzed because plants died).			
^a TNT-equivalents derived from 2-ADNT and 4-ADNT concentrations.			

Table 16. Extractable TNT and TNT-equivalent concentrations in shoots and roots of plants exposed to TNT-amended soil mixtures; concentrations and contents per pot. Mean values and standard deviations are shown (N=3).

TNT Exposure	Final TNT + TNT-Equivalents Plant Tissues				
	(mg kg ⁻¹)		(mg pot ⁻¹)		
	Shoots	Roots	Shoots	Roots	Plant
Grasses					
<i>A. gerardii</i>					
10 mg kg ⁻¹ TNT	0	0	0	0	0
50 mg kg ⁻¹ TNT	0	0	0	0	0
100 mg kg ⁻¹ TNT	90.7 ± 91.3	819.8 ± 91.3	0.02 ± 0.02	0.19 ± 0.17	0.22 ± 0.19
<i>B. gracilis</i>					
10 mg kg ⁻¹ TNT	0	0	0	0	0
50 mg kg ⁻¹ TNT	1,352 ± 111	A	0.31 ± 0.06	A	0.31 ± 0.06
100 mg kg ⁻¹ TNT	8,765 ± 1627	A	1.98 ± 0.28	A	1.98 ± 0.06
<i>E. canadensis</i>					
10 mg kg ⁻¹ TNT	0	0	0	0	0
50 mg kg ⁻¹ TNT	121.0 ± 23.0	0	0.13 ± 0.02	0	0.13 ± 0.02
100 mg kg ⁻¹ TNT	2,632 ± A	A	0.63 ± A	A	0.63 ± A
<i>S. nutans</i>					
10 mg kg ⁻¹ TNT	0	0	0	0	0
50 mg kg ⁻¹ TNT	0	0	0	0	0
100 mg kg ⁻¹ TNT	576.6 ± 157.6	A	0.28 ± 0.26	A	0.28 ± 0.26
<p>Note: Two potential TNT metabolites usually occurred in plants exposed to the target TNT concentration of 100 mg kg⁻¹, one with a short, 2.0 min., and one with a long, 4.2 min., retention time. TNT and TNT metabolite levels in forbs were usually below detection. Exceptions: <i>A. syriaca</i>, in which shoots of two units exposed to 50 mg TNT kg⁻¹ contained 2 and 4 mg kg⁻¹ shoot DW; and in which roots of one unit exposed to 100 mg TNT kg⁻¹ contained 15 mg TNT-equivalents kg⁻¹ root DW. A = absent.</p>					

The known TNT metabolites 2-ADNT and 4-ADNT were usually identified in grasses exposed to the 100-mg kg⁻¹ target TNT concentration. In addition, two unidentified potential TNT metabolites were observed, one with a short, 2.0 min., and one with a longer, 4.2 min., retention time. TNT mass balance estimates of the soil-grass systems were made. The smallest potential TNT loss per 768-g pot content was 11.58 mg in *B. gracilis* vegetated pots amended to the 50-mg kg⁻¹ target TNT level (Table 17). Only a small fraction of the TNT lost from the soils was recovered in the grasses, among which most species lacked the ability to produce viable plants at target TNT levels ≥50 mg kg⁻¹ soil. The only species that survived exposure to the elevated target TNT levels, *A. gerardii*, showed no extractable TNT or TNT metabolites at the 50-mg kg⁻¹ level and accounted for only

Table 17. Extractable TNT and TNT-equivalent mass balances of soil-plant systems. Mean values and standard deviations are shown (N=3).

TNT Exposure	MC Loss from Soil (% initial extractable TNT) ^a	MC Uptake Shoot (% initial extractable TNT) ^a	MC Uptake Root (% initial extractable TNT) ^a	MC Uptake Plant (% initial extractable TNT) ^a	MC Loss Other Processes (% initial extractable TNT) ^a
Grass Tests					
<i>A. gerardii</i>					
10 mg kg ⁻¹ TNT	100	0	0	0	100
50 mg kg ⁻¹ TNT	89.4 ± 3.7	0	0	0	89.4
100 mg kg ⁻¹ TNT	79.0 ± 4.7	0	0.7 ± 0.6	0.8 ± 0.7	78.2
<i>B. gracilis</i>					
10 mg kg ⁻¹ TNT	100	0	0	0	100
50 mg kg ⁻¹ TNT	82.6 ± 2.1	2.2 ± 0.4	A	2.2 ± 0.4	80.4
100 mg kg ⁻¹ TNT	78.9 ± 2.3	6.8 ± 1.0	A	6.8 ± 1.0	72.1
<i>E. Canadensis</i>					
10 mg kg ⁻¹ TNT	100	0	0	0	100
50 mg kg ⁻¹ TNT	87.6 ± 5.8	0.9 ± 0.1	0	0.9 ± 0.1	86.7
100 mg kg ⁻¹ TNT	79.2 ± 0.5	2.2 ± A	A	2.2 ± A	77.0
<i>S. nutans</i>					
10 mg kg ⁻¹ TNT	100	0	0	0	100
50 mg kg ⁻¹ TNT	95.3 ± 0.3	0	0	0	95.3
100 mg kg ⁻¹ TNT	83.0 ± 3.6	1.0 ± 0.9	A	1.0 ± 0.9	82.0
<p>Note: MC = munitions compound; A = absent.</p> <p>^a Initial soil weight: 768 g DW</p> <p>Initial extractable:</p> <p>at target 10 mg kg⁻¹ ≥ 1.83 mg pot⁻¹</p> <p>at target 50 mg kg⁻¹ ≥ 14.02 mg pot⁻¹</p> <p>at target 100 mg kg⁻¹ ≥ 29.23 mg pot⁻¹</p>					

Figure 17. (Concluded).

TNT Exposure	MC Loss from Soil (% initial extractable TNT) ^a	MC Uptake Shoot (% initial extractable TNT) ^a	MC Uptake Root (% initial extractable TNT) ^a	MC Uptake Plant (% initial extractable TNT) ^a	MC Loss Other Processes (% initial extractable TNT) ^a
Forb Tests					
<i>A. retroflexus</i>					
10 mg kg ⁻¹ TNT	100	0	0	0	100
50 mg kg ⁻¹ TNT	88.8 ± 2.2	0	0	0	88.8 ± 2.2
100 mg kg ⁻¹ TNT	98.0	0	0	0	98.0
<i>A. syriaca</i>					
10 mg kg ⁻¹ TNT	100	0	0	0	100
50 mg kg ⁻¹ TNT	99.6 ± 0.4	0	0	0	99.6 ± 0.4
100 mg kg ⁻¹ TNT	34.6 ± 2.9	0	0	0	34.6 ± 2.9
<i>I. lacunose</i>					
10 mg kg ⁻¹ TNT	100	0	0	0	100
50 mg kg ⁻¹ TNT	100	0	0	0	100
100 mg kg ⁻¹ TNT	100	0	0	0	100
<i>P. oleracea</i>					
10 mg kg ⁻¹ TNT	86.4 ± 23.6	0	0	0	86.4 ± 23.6
50 mg kg ⁻¹ TNT	90.0	0	0	0	90.0
100 mg kg ⁻¹ TNT	A	A	A	A	A
<i>S. spinosa</i>					
10 mg kg ⁻¹ TNT	72.3 ± 48.0	0	0	0	72.3 ± 48.0
50 mg kg ⁻¹ TNT	90.3 ± 2.1	0	0	0	90.3 ± 2.1
100 mg kg ⁻¹ TNT	98.8 ± 1.0	0	0	0	98.8 ± 1.0
Note: MC = munitions compound; A = absent.					
^a Initial soil weight: 768 g DW					
Initial extractable:					
at target 10 mg kg ⁻¹ ≥ 0.64 mg pot ⁻¹					
at target 50 mg kg ⁻¹ ≥ 6.24 mg pot ⁻¹					
at target 100 mg kg ⁻¹ ≥ 43.62 mg pot ⁻¹					

0.8 percent of initial at the 100-mg kg⁻¹ level (or 0.22 mg TNT per pot, or 0.2 kg ha⁻¹; Table 16, Table 17). By inference, most TNT loss from the soils may be attributed to processes other than uptake by plants. These processes may include microbial and photochemical degradation in the soil, either or not stimulated by plant exudates, and uptake and metabolism

inside the plants to compounds, possibly conjugates, that were not extracted and detected with the currently used procedures (Burken 2003; Just and Schnoor 2004; Best et al. 2005).

Forbs

The initial extractable TNT concentrations in the amended soils used for the forb tests were also less than the target concentrations and ranged from 0.83 mg kg⁻¹ soil DW (10-mg kg⁻¹ target concentration) to 56.80 mg kg⁻¹ soil (100-mg kg⁻¹ target concentration; Table 14).

The final concentrations of TNT and TNT-equivalents were below detection in the 10-mg kg⁻¹ soils vegetated by three of the five forb species in the forb tests, i.e., by *A. retroflexus*, *A. syriaca*, and *I. lacunosa*, and low in all other soils (Table 15). TNT loss, expressed as percentage of initial, ranged from 72.3 to 100 percent, and was usually greatest in the 10-mg kg⁻¹ soils and least in the 100-mg kg⁻¹ soils – except in soils vegetated with *I. lacunosa*, where TNT and TNT-equivalents were below detection in all soils (Table 15). Total TNT loss, expressed in milligrams TNT per pot, ranged from 0.64 to 43.62 mg (Table 14, Table 15). The greatest absolute TNT loss of 43.62 mg occurred in pots amended to the 100-mg TNT kg⁻¹ target level, which were vegetated by *I. lacunosa*. This would result in a TNT loss of 33.2 kg ha⁻¹.

The final concentrations of TNT and TNT-equivalents accumulated in the forbs were usually below detection (Table 16). Exceptions were found in *A. syriaca*, in which shoots of two units exposed to 50 mg TNT kg⁻¹ contained 2 and 4 mg TNT kg⁻¹ DW, and in which roots of one unit exposed to 100 mg TNT kg⁻¹ contained 15 mg TNT-equivalents kg⁻¹ DW.

TNT mass balance of the soil-forb systems was estimated. The smallest potential TNT loss per 768-g pot content was 5.54 mg (in *A. retroflexus* vegetated pots amended to the 50-mg kg⁻¹ target TNT level; Table 17). Almost none of the TNT lost from the soils was recovered in the forbs, of which one species lacked the ability to produce viable plants at target TNT levels ≥ 50 mg kg⁻¹ soil, i.e., *P. oleracea*. As with the grasses, most TNT loss from the soils may be attributed to processes other than uptake by plants.

RDX exposures

Grasses

The initial extractable RDX concentrations in the amended soils used for the grass tests were less than the target concentrations, and ranged from 5.09 mg kg⁻¹ soil DW (100-mg kg⁻¹ target concentration) to 778.47 mg kg⁻¹ soil (1,000-mg kg⁻¹ target concentration; Table 14). These soils also contained low levels of HMX, because technical RDX served as the source for the amendments.

The final concentrations of RDX were detectable in all soils of the grass tests except the controls (Table 18). The known RDX metabolites, MNX, DNX, and TNX, were below detection. RDX loss, expressed as percentage of initial, ranged from 0 to 78.5 percent, and was greatest in the 100-mg kg⁻¹ soils and least in the 500- and 1,000-mg kg⁻¹ soils (Table 18). Total RDX loss, expressed in milligrams RDX per pot, ranged from 0.59 to 10.76 mg (Tables 14 and 18). The greatest absolute RDX loss of 10.76 mg RDX occurred in pots amended to the 1,000-mg kg⁻¹ target level, which were vegetated by *A. gerardii*. This would result in an RDX loss of 8.2 kg ha⁻¹. RDX accumulated to considerable levels in both roots and shoots of all grasses exposed to soil-based RDX (Table 19). Two accumulation patterns were identified. Accumulation occurred preferably in shoots in *A. gerardii*, *E. canadensis*, and *S. nutans*, but preferably in roots in *B. gracilis*. RDX accumulated most in shoots of *E. canadensis* up to 2,936 mg kg⁻¹ and in roots of *B. gracilis* up to 4,620 mg kg⁻¹, when plants were exposed to the 1,000-mg kg⁻¹ target RDX concentration. The greatest absolute RDX accumulation occurred in *S. nutans* exposed to 1,000 mg RDX kg⁻¹, amounting to 4.50 mg per pot (3.4 kg ha⁻¹). Very low levels of the known RDX metabolite MNX were identified in shoots of *A. gerardii*, *B. gracilis*, and *E. canadensis* plants exposed to the 1,000-mg kg⁻¹ target RDX concentration, while similar levels of DNX and TNX were identified in shoots and roots of *S. nutans* exposed to the latter RDX level. In addition, two potential RDX metabolites were observed, one with a short, 1.6 min., and one with a longer, 4.2 min., retention time.

Table 18. Extractable RDX and HMX concentrations of the remediated soil mixtures at the end of the tests, and calculated loss relative to initial. Mean values and standard deviations are shown (N=3).

RDX Exposure	Final Extractable Explosives Concentration Soil (mg kg ⁻¹ DW)		RDX Loss (% initial extractable RDX)
	RDX	HMX	
Grass Tests			
<i>A. gerardii</i>			
100 mg kg ⁻¹ RDX	1.33 ± 0.44	0.80 ± 0.17	73.8 ± 8.7
500 mg kg ⁻¹ RDX	50.15 ± 7.22	12.53 ± 1.69	0
1,000 mg kg ⁻¹ RDX	764.08 ± 35.71	61.22 ± 11.82	1.8 ± 4.6
<i>B. gracilis</i>			
100 mg kg ⁻¹ RDX	4.32 ± 0.59	1.03 ± 0.17	15.1 ± 11.6
500 mg kg ⁻¹ RDX	72.86 ± 6.31	13.75 ± 3.27	0
1,000 mg kg ⁻¹ RDX	758.15 ± 117.91	59.31 ± 8.68	0
<i>E. canadensis</i>			
100 mg kg ⁻¹ RDX	1.51 ± 0.05	0.87 ± 0.04	70.2 ± 0.9
500 mg kg ⁻¹ RDX	57.17 ± 6.47	13.94 ± 3.49	0
1,000 mg kg ⁻¹ RDX	943.47 ± 77.06	69.48 ± 32.96	0
<i>S. nutans</i>			
100 mg kg ⁻¹ RDX	1.09 ± 0.41	0.86 ± 0.06	78.5 ± 8.1
500 mg kg ⁻¹ RDX	49.28 ± 9.18	13.32 ± 3.72	0
1,000 mg kg ⁻¹ RDX	875.44 ± 54.67	79.84 ± 9.75	0
Forb Tests			
<i>A. retroflexus</i>			
100 mg kg ⁻¹ RDX	7.98 ± 2.10	2.12 ± 0.17	93.3 ± 1.8
500 mg kg ⁻¹ RDX	10.86 ± 0.28	76.11 ± 2.18	78.0 ± 0.6
1,000 mg kg ⁻¹ RDX	66.63 ± 1.31	558.94 ± 20.39	47.7 ± 1.9
<i>A. syriaca</i>			
100 mg kg ⁻¹ RDX	7.27 ± 1.97	2.55 ± 0.27	93.8 ± 1.66
500 mg kg ⁻¹ RDX	87.94 ± 4.38	13.02 ± 0.90	74.6 ± 1.3
1,000 mg kg ⁻¹ RDX	445.10 ± 97.12	56.42 ± 8.40	58.3 ± 9.1
<i>I. lacunosa</i>			
100 mg kg ⁻¹ RDX	5.77 ± 8.02	2.09 ± 0.16	95.1 ± 6.7
500 mg kg ⁻¹ RDX	43.13 ± 18.11	9.97 ± 2.69	87.6 ± 5.2
1,000 mg kg ⁻¹ RDX	442.77 ± 60.39	57.90 ± 7.77	58.58 ± 5.64
<i>P. oleracea</i>			
100 mg kg ⁻¹ RDX	7.54 ± 2.83	2.27 ± 0.56	93.65 ± 2.37
500 mg kg ⁻¹ RDX	71.24 ± 8.97	10.61 ± 0.98	79.5 ± 2.58
1,000 mg kg ⁻¹ RDX	319.69 ± 258.11	39.61 ± 28.73	70.1 ± 24.1
<i>S. spinosa</i>			
100 mg kg ⁻¹ RDX	6.30 ± 1.01	1.72 ± 0.03	94.7 ± 0.9
500 mg kg ⁻¹ RDX	63.63 ± 12.18	9.75 ± 1.31	81.6 ± 3.5
1,000 mg kg ⁻¹ RDX	412.49 ± 78.17	49.62 ± 7.35	61.4 ± 7.3

Table 19. Extractable RDX concentrations in shoots and roots of plants exposed to RDX-amended soil mixtures; concentrations and contents per pot. Mean values and standard deviations are shown (N=3).

RDX Exposure	Final RDX Plant Tissues				
	(mg kg ⁻¹)		(mg pot ⁻¹)		
	Shoots	Roots	Shoots	Roots	Plant
Grasses					
<i>A. gerardii</i>					
100 mg kg ⁻¹ RDX	181.4 ± 32.6	75.5 ± 21.3	0.55 ± 0.07	0.12 ± 0.04	0.67 ± 0.03
500 mg kg ⁻¹ RDX	894.6 ± 153.9	205.9 ± 42.3	2.56 ± 0.68	0.26 ± 0.04	2.82 ± 0.64
1,000 mg kg ⁻¹ RDX	1,438 ± 283; M1	325.0 ± 43.0	3.89 ± 1.43	0.44 ± 0.10	4.33 ± 1.46
<i>B. gracilis</i>					
100 mg kg ⁻¹ RDX	524.4 ± 116.1	1,098 ± 116	0.19 ± 0.08	0.07 ± 0.01	0.26 ± 0.08
500 mg kg ⁻¹ RDX	1,821 ± 238	4,510 ± 924	0.59 ± 0.29	0.30 ± 0.15	0.89 ± 0.34
1,000 mg kg ⁻¹ RDX	1,702 ± 66; M1	4,620 ± 345	0.53 ± 0.19	0.23 ± 0.06	0.75 ± 0.24
<i>E. canadensis</i>					
100 mg kg ⁻¹ RDX	688.3 ± 74.8	172.9 ± 67.8	0.73 ± 0.12	0.10 ± 0.04	0.83 ± 0.10
500 mg kg ⁻¹ RDX	1,924 ± 757	713.6 ± 151.1	2.53 ± 0.67	0.36 ± 0.07	2.89 ± 0.62
1,000 mg kg ⁻¹ RDX	2,936 ± 593; M1	577.7 ± 70.9	2.63 ± 0.60	0.28 ± 0.03	2.91 ± 0.62
<i>S. nutans</i>					
100 mg kg ⁻¹ RDX	163.6 ± 105.0	19.8 ± 12.1	0.38 ± 0.52	0.05 ± 0.05	0.43 ± 0.53
500 mg kg ⁻¹ RDX	801.6 ± 294.0	37.5 ± 8.8	3.09 ± 1.46	0.25 ± 0.03	3.34 ± 1.45
1,000 mg kg ⁻¹ RDX	1,203 ± 688; M2	47.4 ± 21.0; M2	4.10 ± 2.11	0.40 ± 0.11	4.50 ± 2.12
Note: M1 = MNX; M2 = DNX, TNX. Two potential RDX metabolites usually occurred in grasses exposed to target RDX concentrations of 1000 mg kg ⁻¹ , one with a short, 1.6 min., and one with a long, 4.2 min., retention time.					
Forbs					
<i>A. retroflexus</i>					
100 mg kg ⁻¹ RDX	292.5 ± 76.2	329.3 ± 144.8	1.27 ± 0.39	0.34 ± 0.14	1.61 ± 0.53
500 mg kg ⁻¹ RDX	1,284 ± 141	869.3 ± 432.8	3.55 ± 0.36	0.84 ± 0.51	4.39 ± 0.45
1,000 mg kg ⁻¹ RDX	1,154 ± 136; M1	1,054 ± 376; M1	4.07 ± 1.79	0.92 ± 0.55	4.99 ± 1.57
<i>A. syriaca</i>					
100 mg kg ⁻¹ RDX	297.1 ± 98.0	48.2 ± 10.78	0.75 ± 0.27	0.09 ± 0.02	0.84 ± 0.27
500 mg kg ⁻¹ RDX	525.1 ± 438.4	161.5 ± 142.5	1.36 ± 1.17	0.22 ± 0.19	1.58 ± 1.36
1,000 mg kg ⁻¹ RDX	1,183 ± 365	554.8 ± 135.7	2.05 ± 0.90	0.44 ± 0.09	2.49 ± 0.87
<i>I. lacunosa</i>					
100 mg kg ⁻¹ RDX	75.9 ± 33.7	41.8 ± 28.6	1.16 ± 0.42	0.23 ± 0.13	1.40 ± 0.54
500 mg kg ⁻¹ RDX	189.6 ± 20.5	116.3 ± 34.4	2.28 ± 0.25	0.37 ± 0.12	2.65 ± 0.35
1,000 mg kg ⁻¹ RDX	578.8 ± 44.4	240.3 ± 47.4	7.56 ± 0.41	0.86 ± 0.11	8.42 ± 0.46
<i>P. oleracea</i>					
100 mg kg ⁻¹ RDX	185.4 ± 41.3	72.4 ± 23.8	0.64 ± 0.17	0.06 ± 0.04	0.70 ± 0.19
500 mg kg ⁻¹ RDX	389.2 ± 70.6	162.2 ± 28.3	1.10 ± 0.07	0.15 ± 0.04	1.24 ± 0.09
1,000 mg kg ⁻¹ RDX	636.4 ± 134.4	625.9 ± 524.3	2.21 ± 0.61	0.86 ± 1.07	3.07 ± 1.30
<i>S. spinosa</i>					
100 mg kg ⁻¹ RDX	46.4 ± 19.7	48.6 ± 9.0	0.19 ± 0.04	0.07 ± 0.02	0.25 ± 0.03
500 mg kg ⁻¹ RDX	203.8 ± 97.6	96.8 ± 16.9	0.57 ± 0.39	0.13 ± 0.08	0.70 ± 0.33
1,000 mg kg ⁻¹ RDX	572.3 ± 124.3	181.3 ± 36.6	2.43 ± 1.11	0.21 ± 0.08	2.64 ± 1.14
Note: M1 = MNX. No other potential RDX metabolites observed.					

RDX mass balance of the soil-grass systems was estimated. The smallest potential RDX loss per 768-g pot content was 0.59 mg RDX (in *B. gracilis* vegetated pots amended to the lowest 100-mg kg⁻¹ target RDX level; Table 20). As with TNT, only a small fraction of the RDX lost from the soils was recovered in the grasses, all of which (except *E. trichoides*) produced viable plants up to the greatest 1,000-mg kg⁻¹ target RDX level. The greatest amount was recovered in *S. nutans* and accounted for only 0.7 percent of initial (or 4.50 mg RDX per pot; Tables 18 and 20). As with TNT, most RDX loss from the soils may be attributed to processes other than uptake by plants.

Forbs

The initial extractable RDX concentrations in the amended soils used for the forb tests were greater than the 100- and 1,000-mg kg⁻¹ target concentrations, less than the 500-mg kg⁻¹ target concentration, and ranged from 119.00 mg kg⁻¹ soil DW (100 mg kg⁻¹ target concentration) to 1069.07 mg kg⁻¹ soil (1,000 mg kg⁻¹ target concentration; Table 14).

The final RDX concentrations were detectable in all soils of the forb tests except the controls, and the RDX metabolites MNX, DNX, and TNX, were below detection (Table 18). RDX loss, expressed as percentage of initial, ranged from 47.4 to 95.1 percent, and was greatest in the 100-mg kg⁻¹ soils and least in the 1,000-mg kg⁻¹ soils (Table 18). Total RDX loss, expressed in milligrams RDX per pot, ranged from 85.27 to 575.56 mg (Tables 14 and 18).

The greatest absolute RDX loss of 575.56 mg RDX occurred in pots amended to the 1,000-mg kg⁻¹ target level, which were vegetated by *P. oleracea*. This would result in a RDX loss of 437 kg ha⁻¹.

RDX accumulated to considerable levels in both roots and shoots of all forbs exposed to soil-based RDX (Table 19). Two accumulation patterns were identified. RDX accumulated preferably in shoots in two forb species, i.e., *A. syriaca* and *P. oleracea*. This pattern is identical to one of the accumulation patterns found in the grasses. RDX accumulated to similar levels in roots and shoots in three other forb species. This pattern occurred in *A. retroflexus* plants upon exposure to all RDX levels, and in *I. lacunosa* and *S. spinosa* plants upon exposure to 100-mg RDX kg⁻¹ and 500-mg RDX kg⁻¹ levels. Both latter plant species exhibited RDX accumulation preferably in shoots at the higher 1,000 mg kg⁻¹ RDX exposure level.

Table 20. Extractable RDX mass balances of soil-plant systems. Mean values and standard deviations are shown (N=3).

RDX Exposure	MC Loss from Soil (% initial extractable RDX)	MC Uptake Shoot (% initial extractable RDX)	MC Uptake Root (% initial extractable RDX)	MC Uptake Plant (% initial extractable RDX)	MC Loss Other Processes (% initial extractable RDX)
Grass Tests					
<i>A. gerardii</i>					
100 mg kg ⁻¹ RDX	73.8 ± 8.7	14.0 ± 1.8	3.1 ± 1.1	17.1 ± 0.7	56.7
500 mg kg ⁻¹ RDX	0	7.0 ± 1.9	0.7 ± 0.1	7.7 ± 1.8	0
1,000 mg kg ⁻¹ RDX	1.8 ± 4.6	0.7 ± 0.2	0.0 ± 0.0	0.7 ± 0.2	1.1
<i>B. gracilis</i>					
100 mg kg ⁻¹ RDX	15.1 ± 11.6	4.8 ± 1.9	1.8 ± 0.1	6.6 ± 2.0	8.5
500 mg kg ⁻¹ RDX	0	1.6 ± 0.8	0.8 ± 0.4	2.4 ± 0.9	0
1,000 mg kg ⁻¹ RDX	0	0.1 ± 0.0	0	0.1 ± 0.0	0
<i>E. canadensis</i>					
100 mg kg ⁻¹ RDX	70.2 ± 0.9	18.5 ± 3.2	2.6 ± 0.9	21.1 ± 2.4	49.1
500 mg kg ⁻¹ RDX	0	6.9 ± 1.8	1.0 ± 0.2	7.9 ± 1.7	0
1,000 mg kg ⁻¹ RDX	0	0.4 ± 0.1	0.0 ± 0.0	0.4 ± 0.1	0
<i>S. nutans</i>					
100 mg kg ⁻¹ RDX	78.5 ± 8.1	9.7 ± 13.3	1.4 ± 1.4	11.1 ± 13.6	67.4
500 mg kg ⁻¹ RDX	0	8.5 ± 4.0	0.7 ± 0.1	9.2 ± 4.0	0
1,000 mg kg ⁻¹ RDX	0	0.7 ± 0.0	0.0 ± 0.0	0.7 ± 0.0	0
<p>Note: MC = munitions compound; A = absent. ^a Initial soil weight: 768 g DW Initial extractable: at target 100 mg kg⁻¹ ≥ 3.91 mg pot⁻¹ at target 500 mg kg⁻¹ ≥ 36.39 mg pot⁻¹ at target 1,000 mg kg⁻¹ ≥ 597.50 mg pot⁻¹</p>					

Table 20. (Concluded)

RDX Exposure	MC Loss from Soil (% initial extractable RDX)	MC Uptake Shoot (% initial extractable RDX)	MC Uptake Root (% initial extractable RDX)	MC Uptake Plant (% initial extractable RDX)	MC Loss Other Processes (% initial extractable RDX)
Forb Tests					
<i>A. retroflexus</i>					
100 mg kg ⁻¹ RDX	93.3 ± 1.8	1.4 ± 0.4	0.4 ± 0.2	1.8 ± 0.6	91.5
500 mg kg ⁻¹ RDX	78.0 ± 0.6	1.3 ± 0.1	0.3 ± 0.2	1.7 ± 0.2	76.3
1,000 mg kg ⁻¹ RDX	47.7 ± 1.9	0.5 ± 0.2	0.1 ± 0.1	0.6 ± 0.2	47.1
<i>A. syriaca</i>					
100 mg kg ⁻¹ RDX	93.8 ± 1.66	0.8 ± 0.3	0.1 ± 0.0	0.9 ± 0.3	93.1
500 mg kg ⁻¹ RDX	74.6 ± 1.3	0.5 ± 0.4	0.1 ± 0.1	0.6 ± 0.5	74.0
1,000 mg kg ⁻¹ RDX	58.3 ± 9.1	0.3 ± 0.1	0.1 ± 0.0	0.4 ± 0.1	57.9
<i>I. lacunosa</i>					
100 mg kg ⁻¹ RDX	95.1 ± 6.7	1.3 ± 0.5	0.3 ± 0.1	1.6 ± 0.6	93.5
500 mg kg ⁻¹ RDX	87.6 ± 5.2	0.9 ± 0.1	0.1 ± 0.0	1.0 ± 0.1	86.6
1,000 mg kg ⁻¹ RDX	58.6 ± 5.64	0.9 ± 0.1	0.1 ± 0.0	1.0 ± 0.1	57.6
<i>P. oleracea</i>					
100 mg kg ⁻¹ RDX	93.7 ± 2.4	0.7 ± 0.2	0.1 ± 0.0	0.8 ± 0.2	92.9
500 mg kg ⁻¹ RDX	79.5 ± 2.6	0.4 ± 0.0	0.1 ± 0.0	0.5 ± 0.0	79.0
1,000 mg kg ⁻¹ RDX	70.1 ± 24.1	0.3 ± 0.0	0.1 ± 0.1	0.4 ± 0.2	69.7
<i>S. spinosa</i>					
100 mg kg ⁻¹ RDX	94.7 ± 0.9	0.2 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	94.4
500 mg kg ⁻¹ RDX	81.6 ± 3.5	0.2 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	81.3
1,000 mg kg ⁻¹ RDX	61.4 ± 7.3	0.3 ± 0.1	0.0 ± 0.0	0.3 ± 0.1	61.1
Note: MC = munitions compound; A = absent. ^a Initial soil weight: 768 g DW Initial extractable: at target 100 mg kg ⁻¹ → 91.39 mg pot ⁻¹ at target 500 mg kg ⁻¹ → 266.28 mg pot ⁻¹ at target 1,000 mg kg ⁻¹ → 821.05 mg pot ⁻¹					

RDX accumulated most in shoots up to 1,284 mg kg⁻¹ when *A. retroflexus* plants were exposed to the 500-mg kg⁻¹ target RDX concentration, and in roots up to 1,059 mg kg⁻¹ when plants were exposed to the 1,000-mg kg⁻¹ target RDX concentration. The greatest absolute RDX accumulation occurred in *I. lacunosa* exposed to 1,000 mg RDX kg⁻¹, amounting to 8.42 mg per pot (6.4 kg ha⁻¹). The only RDX degradation compound identified, MNX, occurred in shoots and roots of *A. retroflexus* exposed to the 1,000-mg kg⁻¹ target RDX concentration. No other potential RDX metabolites were observed.

RDX mass balance of the soil-forb systems was estimated. The smallest potential RDX loss per 768-g pot contents was 85.27 mg RDX (in *A. retroflexus* vegetated pots amended to the lowest 100-mg kg⁻¹ RDX target level (Table 20). As with TNT, only a small fraction of the RDX lost from the soils was recovered in the forbs, of which all species produced viable plants up to the greatest 1,000-mg kg⁻¹ target RDX level. The greatest amount was recovered in *I. lacunosa* and accounted only for 1.0 percent of initial (or 8.42 mg RDX per pot; Tables 18 and 20). As with TNT, most RDX loss from the soils may be attributed to processes other than uptake by plants.

4 Discussion

In the current experiments, energetics loss from soils due to vegetation by various plant species was quantified and differences associated with differences in plant species characteristics were distinguished. Unplanted controls were not included in the experiments, and, therefore, energetics loss from soils in the absence of vegetation was not quantified.

The growth response of eight of the ten species included in this study was considerable. The growth response of the remaining two species (*B. gracilis* and *E. trichoides*), both grasses, was very low; and overall germination was poor for *E. trichoides*.

Mass balance was calculated relative to the initial extractable energetics concentrations. Because the initial extractable energetics concentrations in the soil mixtures deviated in different ways from the target TNT and RDX levels in the grass and forb experiments, respectively, comparison of phytoremediation characteristics between species within the grasses and within forbs tested is more relevant than comparison between grasses and forbs. For instance, the initial extractable soil RDX concentrations were far lower in the grass experiment than in the forb experiment. In addition, the RDX mass balances for these soil-plant units were less accurate than for the soil-plant units exposed to the lowest (100-mg kg⁻¹) target RDX level because of the variability in the initial extractable medium and maximum soil RDX levels (Table 21).

Phytoremediation of TNT in soil-plant systems

TNT was toxic to plants at lower concentrations than RDX, but plant response varied strongly with species. Among the grasses, four of the five species (*B. gracilis*, *E. canadensis*, *E. trichoides*, and *S. nutans*), and among the forbs, one species (*P. oleracea*), lacked the ability to produce viable plants at target TNT levels > 50 mg kg⁻¹ soil. One grass and one forb were the most tolerant to TNT, i.e., *A. gerardii* and *I. lacunosa*, which both tolerated the target TNT level of 100 mg kg⁻¹ (Table 21).

Table 21. Plant tolerance, uptake, and capacity to metabolize maximum loss from soil due to potential plant-assisted capacity of TNT and RDX, and evapotranspiration rates (grasses only) based on the results of the grass and forb tests.

Plant Species	Tolerance	TNT Uptake	TNT Metabolism	Max. TNT Loss from Soil ¹ (mg pot ⁻¹)	Evapotranspiration (L m ⁻² d ⁻¹)
TNT Exposures					
Grasses					
<i>A. gerardii</i>	≤100 mg kg ⁻¹	+	+	22.86	1.7-2.6
<i>B. gracilis</i>	<50 mg kg ⁻¹	+	+	21.07	1.3-1.6
<i>E. canadensis</i>	<50 mg kg ⁻¹	+	+	22.51	1.4-2.1
<i>S. nutans</i>	≤50 mg kg ⁻¹	+	+	23.97	1.7-2.2
Forbs					
<i>A. retroflexus</i>	≤50 mg kg ⁻¹	BD	±	42.75	
<i>A. syriaca</i>	≤50 mg kg ⁻¹	BD	BD	15.09	
<i>I. lacunosa</i>	≤100 mg kg ⁻¹	BD	BD	43.62	
<i>P. oleracea</i>	<50 mg kg ⁻¹	BD	BD	5.62 ³	
<i>S. spinosa</i>	≤50 mg kg ⁻¹	BD	BD	43.10	
RDX Exposures					
Grasses					
<i>A. gerardii</i>	≥1,000 mg kg ⁻¹	+	+	≥2.22	2.4
<i>B. gracilis</i>	≥1,000 mg kg ⁻¹	+	+	≥0.33	1.2-1.4
<i>E. canadensis</i>	≥1,000 mg kg ⁻¹	+	+	≥1.95	2.0-2.2
<i>S. nutans</i>	≥1,000 mg kg ⁻¹	+	+	≥2.64	2.0-2.2
Forbs					
<i>A. retroflexus</i>	≥1,000 mg kg ⁻¹	+	+	≥83.62	
<i>A. syriaca</i>	≥1,000 mg kg ⁻¹	+	BD	≥85.08	
<i>I. lacunosa</i>	≥1,000 mg kg ⁻¹	+	BD	≥85.45	
<i>P. oleracea</i>	≥1,000 mg kg ⁻¹	+	BD	≥84.90	
<i>S. spinosa</i>	≥1,000 mg kg ⁻¹	+	BD	≥86.27	
Note: BD = below detection. ¹ Calculated for 100-mg kg ⁻¹ target TNT soils. ² Calculated for 100-mg kg ⁻¹ target RDX soils. ³ Calculated for 50-mg kg ⁻¹ target TNT soils.					

Classified as TNT-tolerant (using 50-mg TNT kg⁻¹ target soil as criterion) are the following:

- Among the grasses: *A. gerardii*, *S. nutans*
- Among the forbs: *A. retroflexus*, *A. syriaca*, *I. lacunosa*, *S. spinosa*

Total TNT loss from the soil-plant systems was considerable. The greatest losses from soil were found in grass-vegetated units of 18.4 kg TNT ha⁻¹ with *S. nutans* present, and in forb-vegetated units of 33.2 kg ha⁻¹ with *I. lacunosa* present (Table 17).

Classified in order of association with the greatest TNT loss from soil due to other processes than plant uptake alone are (using 100-mg TNT kg⁻¹ soil mass balance as criterion; Table 21) the following:

- Among the grasses: *S. nutans* > *A. gerardii* > *E. canadensis* > *B. gracilis*
- Among the forbs: *I. lacunosa* > *S. spinosa* > *A. retroflexus* > *A. syriaca* > *P. oleracea*.

Only a very small fraction or none of the TNT dose was recovered in the plant materials themselves. TNT and TNT metabolites were identified in four of the five grass species. The greatest accumulation of TNT + TNT-equivalents in grasses was found in *A. gerardii* (shoots and roots) amounting to 0.2 kg ha⁻¹. Accumulation of TNT and TNT metabolites in forbs was usually below detection. Within forbs, the only exceptions were selected plants of *A. syriaca*, where low levels of TNT were found in the shoots of one replicate and low levels of 2-ADNT and 4-ADNT were found in the roots of a second replicate.

Classified as TNT uptaker and degrader are (Table 21) as follows:

- Among the grasses: *A. gerardii*, *B. gracilis*, *E. canadensis*, *S. nutans*
- Among the forbs: *A. retroflexus* (potentially)

Thus, most TNT loss from soils, ranging from 80.4 to 99.6 percent (Table 17) or 18.4 to 33.0 kg ha⁻¹ (Table 21), may be attributed to processes other than uptake by plants. These processes may include microbial degradation, photochemical degradation, both potentially stimulated by plant exudates, and uptake and metabolism inside the plants to compounds, possibly conjugates, that were not extracted and detected with the currently used procedures.

Besides energetics tolerance, resilience, and energetics uptake and metabolism, plant traits such as a high evapotranspiration rate and critical root diameter may contribute to an increased phytoremediation capacity. A high evapotranspiration rate is expected to stimulate upward transport of

energetics in the vadose zone of the soil towards the vegetation and transport within the plant itself.

Classified in order of evapotranspiration rate are (Table 21) the following:

- Among the grasses: *A. gerardii* > *S. nutans* > *E. canadensis* > *B. gracilis*

A critical root diameter of 0.38 to 0.59 mm was identified in grasses; this diameter would allow species-characteristic full root length to be reached upon exposure to elevated TNT and RDX levels. No critical root diameter was identified in the forbs. Since root length is an important determinant of the soil area impacted by a plant, long roots are expected to increase the plant-impacted area.

Classified in order of specific root length are (Tables 8 and 11) the following:

- Among the grasses: *B. gracilis* > *E. canadensis* > *A. gerardii* > *S. nutans*
- Among the forbs: *I. lacunosa* > *S. spinosa* > *A. syriaca* > *A. retroflexus* > *P. oleracea*

Phytoremediation of RDX in soil-plant systems

All plants tolerated RDX up to the target level of 1,000 mg kg⁻¹ (Table 21).

Classified as RDX-tolerant (using 1,000 mg RDX kg⁻¹ target soil as criterion) are:

- Among the grasses: *A. gerardii*, *B. gracilis*, *E. canadensis*, *S. nutans*
- Among the forbs: *A. retroflexus*, *A. syriaca*, *I. lacunosa*, *P. oleracea*, *S. spinosa*

Total RDX loss from the soil-plant systems ranged from 8.2 kg ha⁻¹ in units vegetated by *A. gerardii* to 437 kg ha⁻¹ in units vegetated by *P. oleracea* (Table 20).

Classified in order of association with the greatest RDX loss from soil due to other processes than plant uptake alone are (using 100 mg RDX kg⁻¹ soil mass balance as criterion; Table 21) the following:

- Among the grasses: *S. nutans*> *A. gerardii*> *E. canadensis*> *B. gracilis*
- Among the forbs: *S. spinosa*> *I. lacunosa*> *A. syriaca*> *P. oleracea*> *A. retroflexus*

As with TNT, only a very small fraction of the RDX dose was recovered in the plant materials themselves. The greatest RDX accumulation was found for grasses in *S. nutans*, amounting to 3.4 kg ha⁻¹; very low levels of DNX and TNX were also identified in roots and shoots of this species. A different RDX metabolite, MNX, was found in the shoots of three other grasses, i.e., *A. gerardii*, *B. gracilis*, and *E. canadensis*. The greatest RDX accumulation in forbs was found in *I. lacunosa*, i.e., 6.4 kg ha⁻¹. Only one RDX metabolite, MNX, was identified, and this occurred in shoots and roots of *A. retroflexus*.

Classified in order of RDX uptake are (Table 19) the following:

- Among the grasses: *S. nutans*> *A. gerardii*> *E. canadensis*> *B. gracilis*
- Among the forbs: *I. lacunosa*> *A. retroflexus*> *P. oleracea*> *S. spinosa*> *A. syriaca*

Classified as RDX degraders are (Table 21) the following:

- Among the grasses: *A. gerardii*, *B. gracilis*, *E. canadensis*, *S. nutans*
- Among the forbs: *A. retroflexus*

Thus, as with TNT, most RDX loss from soils, ranging from 0 to 94.4 percent (Table 20) or 0 to 437 kg ha⁻¹ may be attributed to processes other than uptake by plants. These processes may include microbial degradation, photochemical degradation, both potentially stimulated by plant exudates, and uptake and metabolism inside the plants to compounds, possibly conjugates, that were not extracted and detected with the currently used procedures.

Conclusions and recommendations for research

1. Of the ten plant species tested, two grasses (*A. gerardii* and *S. nutans*) and four forbs (*A. retroflexus*, *A. syriaca*, *I. lacunosa*, and *S. spinosa*) were classified as TNT-tolerant.
2. Total TNT loss from soil by processes other than plant TNT uptake ranged from 18.4 to 33.2 kg TNT ha⁻¹ in grasses and forbs, respectively. TNT loss decreased in the order *S. nutans*> *A. gerardii*> *E. canadensis*> *B. gracilis*

- in grasses; and in the order *I. lacunosa* > *S. spinosa* > *A. retroflexus* > *A. syriaca* > *P. oleracea* in forbs.
3. Plant TNT uptake ranged from 0.2 kg ha⁻¹ in grasses to almost zero in forbs.
 4. Four grasses took up and metabolized TNT, i.e., *A. gerardii*, *B. gracilis*, *E. canadensis* and *S. nutans*. One forb showed some potential for TNT uptake and metabolism, i.e., *A. retroflexus*.
 5. All plant species were classified as RDX-tolerant.
 6. Total RDX loss from soil by processes other than plant RDX uptake ranged from 8.2 to 437 kg RDX ha⁻¹ in grasses and forbs, respectively. RDX-loss decreased in the order *S. nutans* > *A. gerardii* > *E. canadensis* > *B. gracilis* in grasses; and in the order *S. spinosa* > *I. lacunosa* > *A. syriaca* > *P. oleracea* > *A. retroflexus* in forbs.
 7. Plant RDX uptake ranged from 3.4 kg ha⁻¹ in grasses to 6.4 kg ha⁻¹ in forbs. RDX uptake decreased in the order *S. nutans* > *A. gerardii* > *E. canadensis* > *B. gracilis* in grasses; and in the order *I. lacunosa* > *A. retroflexus* > *P. oleracea* > *S. spinosa* > *A. syriaca* in forbs.
 8. Four grasses metabolized RDX, i.e., *A. gerardii*, *B. gracilis*, *E. canadensis* and *S. nutans*. One forb metabolized RDX, i.e., *A. retroflexus*.

Two grass and one forb species were identified as showing potential for successful phytoextraction, in-plant degradation, and plant-assisted phytoremediation of TNT and RDX in contaminated soil, i.e., *S. nutans*, *A. gerardii*, and *A. retroflexus*. Criteria for this identification were TNT- and RDX-tolerant, associated with considerable loss of TNT and RDX from soil, with the ability to take up and metabolize both TNT and RDX; other characteristics included intermediate to high evapotranspiration rates and relatively short specific root lengths. These plants are recommended for further quantitative study of phytoextraction capacity in scaled-up systems.

Three other forb species were identified as showing potential for successful phytostabilization and, possibly, plant-assisted phytoremediation of TNT and RDX, i.e., *A. syriaca*, *I. lacunosa*, and *S. spinosa*. Particularly *I. lacunosa* with its great specific root length appears to be a suitable candidate. Criteria for this identification were TNT- and RDX-tolerant, associated with considerable loss of TNT and RDX from soil, with the ability to take up RDX. These plants are recommended for further quantitative study of phytostabilization capacity in scaled-up systems.

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Appendix A: Documented Geographic Distribution of Selected Herbaceous Plant Species at Military Installations in the Continental United States

Background

For a variety of reasons, including those related to site remediation and restoration, Army and other military land and facility managers need to know of expected or actual distribution of plant species at installations used for training and testing purposes.

The ongoing SERDP Research Project ER-1500, titled “Plant-based Containment/Treatment of Energetic Material Releases for Application on Testing and Training Ranges,” has an objective to explore the potential for utilizing plant-based biological processes to reduce or eliminate environmental effects of military munitions (i.e., explosives) compound residues. As part of this project, information on several hundred herbaceous plant species was reviewed and laboratory experiments are conducted on a selection of these plants. Based on results of the review and experiments, in part reported previously (Best et al. 2007) and herein, 17 plant species were identified which may have broader utility on military ranges for explosives residue remediation.

The 16 species listed in Table A1 were included in short-term screening experiments for tolerance towards TNT and RDX. An additional species identified, but not further tested, is *Abutilon avicennae* (Velvet leaf; synonymous to *A. theophrasti*). Our studies focus on native herbaceous plant species. However, it is recognized that introduced and other non-native species may have application potential also. Consequently, species that have been introduced and become established outside their native range (i.e., *Datura stramonium* and *Abutilon avicennae*) are included in the current geographic distribution overview of the identified species.

Table A1. Herbaceous plant species included in short-term screening experiments for tolerance towards TNT and RDX.

Plant Species Name	Common Name
Grass	
<i>Achnatherum hymenoides</i>	Indian ricegrass
<i>Agropyron smithii</i>	Western wheatgrass
<i>Andropogon gerardii</i>	Big bluestem
<i>Bouteloua gracilis</i>	Blue grama
<i>Elymus canadensis</i>	Canadian wild rye
<i>Eragrostis trichoides</i>	Sand lovegrass
<i>Panicum virgatum</i>	Switchgrass
<i>Sorghastrum nutans</i>	Indiangrass
Forb	
<i>Achillea millefolium</i>	Common yarrow
<i>Amaranthus retroflexus</i>	Redroot pigweed
<i>Asclepias syriaca</i>	Common milkweed
<i>Datura stramonium</i>	Jimson weed
<i>Ipomoea lacunosa</i>	Morning glory
<i>Portulaca oleracea</i>	Common purslane
<i>Polygonum pensylvanicum</i>	Pennsylvania smartweed
<i>Sida spinosa</i>	Prickly sida

Plant distributions at state, county, and installation scales

Species distributions depend on species characteristics and environmental factors. Among the environmental factors climate, soil, moisture, and nutrient status are the most important. Thus, although a species could persist in a particular geographic or political boundary region, it may be limited by certain ecological conditions, and, therefore, species distributions are not necessarily uniform within a delineated boundary.

The distribution of vascular plant species within the United States and its territories is reported in the U.S. Department of Agriculture (USDA), Natural Resources Conservation Service, Plants Database (<http://plants.usda.gov>), following a standardized format. While it provides the most comprehensive overview currently available, this database is not complete. For example, jimson weed has not been reported to occur in Wyoming (Figure A12), but since it has been reported for all adjacent states, the likelihood of occurrence in Wyoming is high.

This database also provides information on species occurrence at the county level. Presence of a plant species in a county automatically is translated in presence of that species in the state in which the county is located. This can be misleading, since as mentioned above, plant species distribution may be more localized.

The locations of Army installations, including some Army National Guard properties, and associated training and testing ranges, were identified from available sources. Sources included the ERDC file data and data from Environmental Systems Research Institute (ESRI) ArcGIS (<http://esri.com>). This is not a full listing, as different sources compile installation information differently. For example, many National Guard properties are considered state owned and therefore are not shown on U.S. Department of Defense listings. Also, because of Base Realignment and Closure (BRAC) actions, the activity and ownership status of other installations is uncertain. Nonetheless, the listing provided herein is reasonably complete. The location of each installation by county was matched with the information of plant species distribution contained in the USDA database. The matching results are presented in Table A2. This approach indicates a high likelihood for a particular plant species to occur at a given installation, but it does not confirm its presence. However, since plant species surveys for installations and county distribution information of vascular plants in the USDA database are incomplete, and installation plant survey data of installations are not readily available or exceed the scope of this geographic distribution overview of the 17 identified plant species, this approach provides the most informative overview possible at this time. The geographic distributions of the 17 selected species are shown in Figures A1 through A17. The data contained in Table A2 are shown in map form in Figure A18.

Reference

- Best, E. P. H., T. Smith, F. L. Hagen, J. Dawson, and A. J. Torrey. 2007. *Candidate herbaceous plants for phytoremediation of energetics on ranges*. ERDC TR-07-11. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

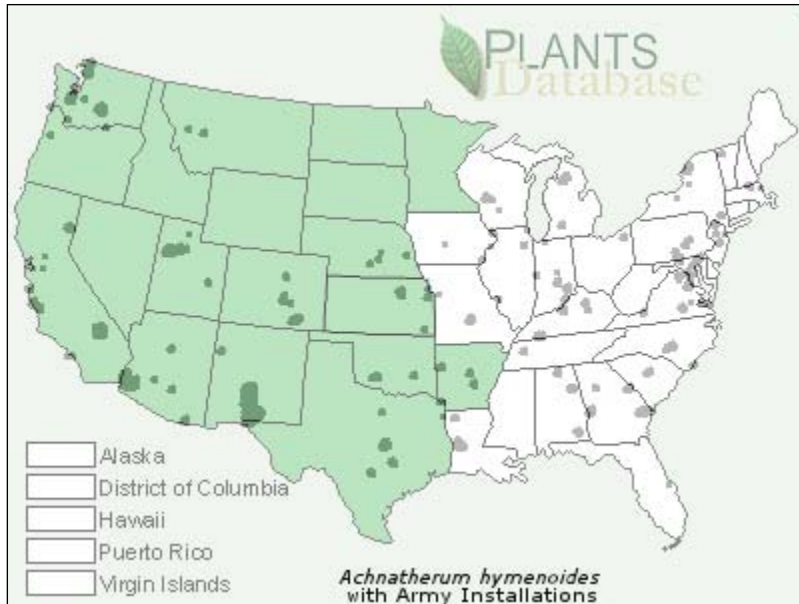


Figure A1. Map of the continental United States with locations of Army installations marked by opaque spots. Green areas represent the distribution of *Achnatherum hymenoides* documented in the USDA database.



Figure A2. Map of the continental United States with locations of Army installations marked by opaque spots. Green areas represent the distribution of *Agropyron smithii* documented in the USDA database.



Figure A3. Map of the continental United States with locations of Army installations marked by opaque spots. Green areas represent the distribution of *Andropogon gerardii* documented in the USDA database.

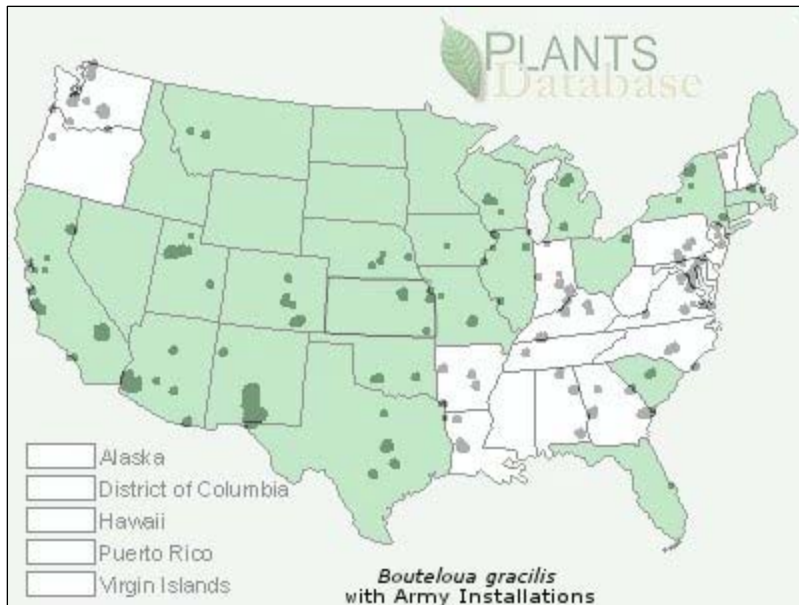


Figure A4. Map of the continental United States with locations of Army installations marked by opaque spots. Green areas represent the distribution of *Bouteloua gracilis* documented in the USDA database.

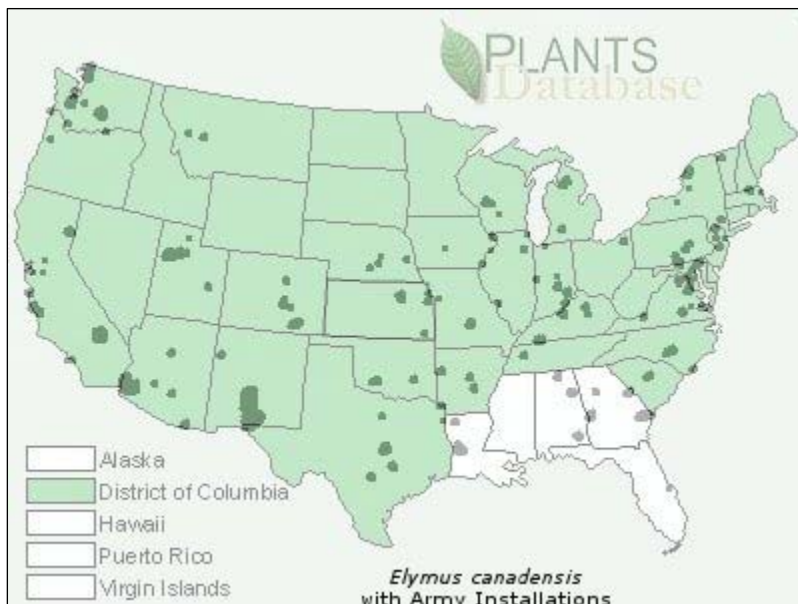


Figure A5. Map of the continental United States with locations of Army installations marked by opaque spots. Green areas represent the distribution of *Elymus canadensis* documented in the USDA database.

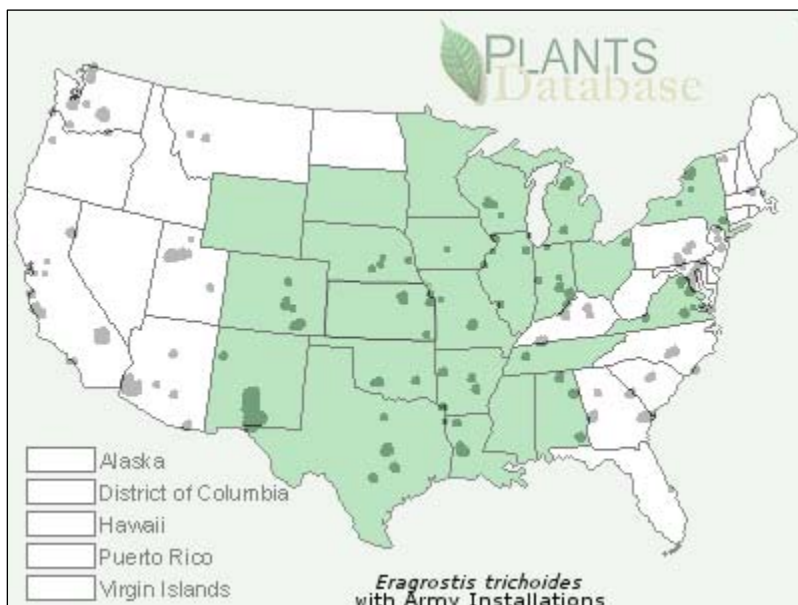


Figure A6. Map of the continental United States with locations of Army installations marked by opaque spots. Green areas represent the distribution of *Eragrostis trichoides* documented in the USDA database.



Figure A7. Map of the continental United States with locations of Army installations marked by opaque spots. Green areas represent the distribution of *Panicum virgatum* documented in the USDA database.



Figure A8. Map of the continental United States with locations of Army installations marked by opaque spots. Green areas represent the distribution of *Sorghastrum nutans* documented in the USDA database.



Figure A9. Map of the continental United States with locations of Army installations marked by opaque spots. Green areas represent the distribution of *Achillea millefolium* documented in the USDA database.



Figure A10. Map of the continental United States with locations of Army installations marked by opaque spots. Green areas represent the distribution of *Amaranthus retroflexus* documented in the USDA database.



Figure A11. Map of the continental United States with locations of Army installations marked by opaque spots. Green areas represent the distribution of *Asclepias syriaca* documented in the USDA database.

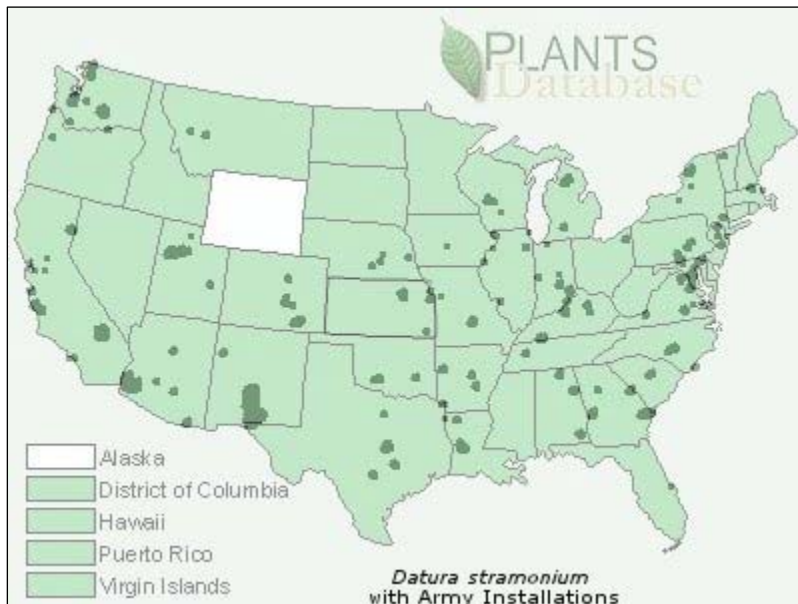


Figure A12. Map of the continental United States with locations of Army installations marked by opaque spots. Green areas represent the distribution of *Datura stramonium* documented in the USDA database.

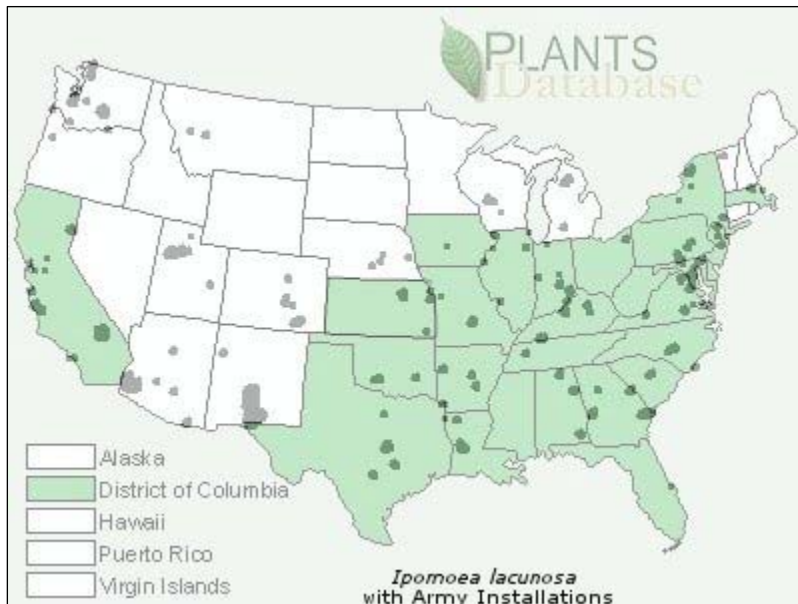


Figure A13. Map of the continental United States with locations of Army installations marked by opaque spots. Green areas represent the distribution of *Ipomoea lacunosa* documented in the USDA database.



Figure A14. Map of the continental United States with locations of Army installations marked by opaque spots. Green areas represent the distribution of *Portulaca oleracea* documented in the USDA database.

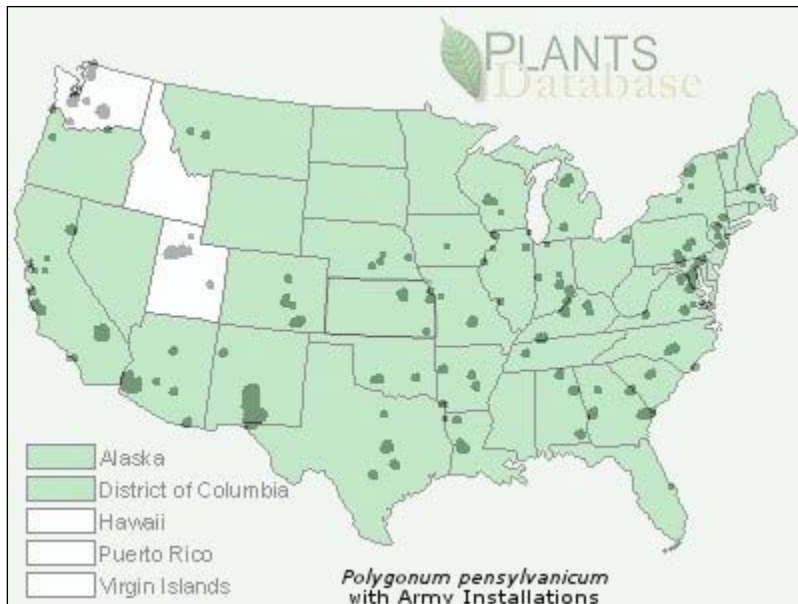


Figure A15. Map of the continental United States with locations of Army installations marked by opaque spots. Green areas represent the distribution of *Polygonum pensylvanicum* documented in the USDA database.



Figure A16. Map of the continental United States with locations of Army installations marked by opaque spots. Green areas represent the distribution of *Sida spinosa* documented in the USDA database.



Figure A17. Map of the continental United States with locations of Army installations marked by opaque spots. Green areas represent the distribution of *Abutilon avicennae* documented in the USDA database.

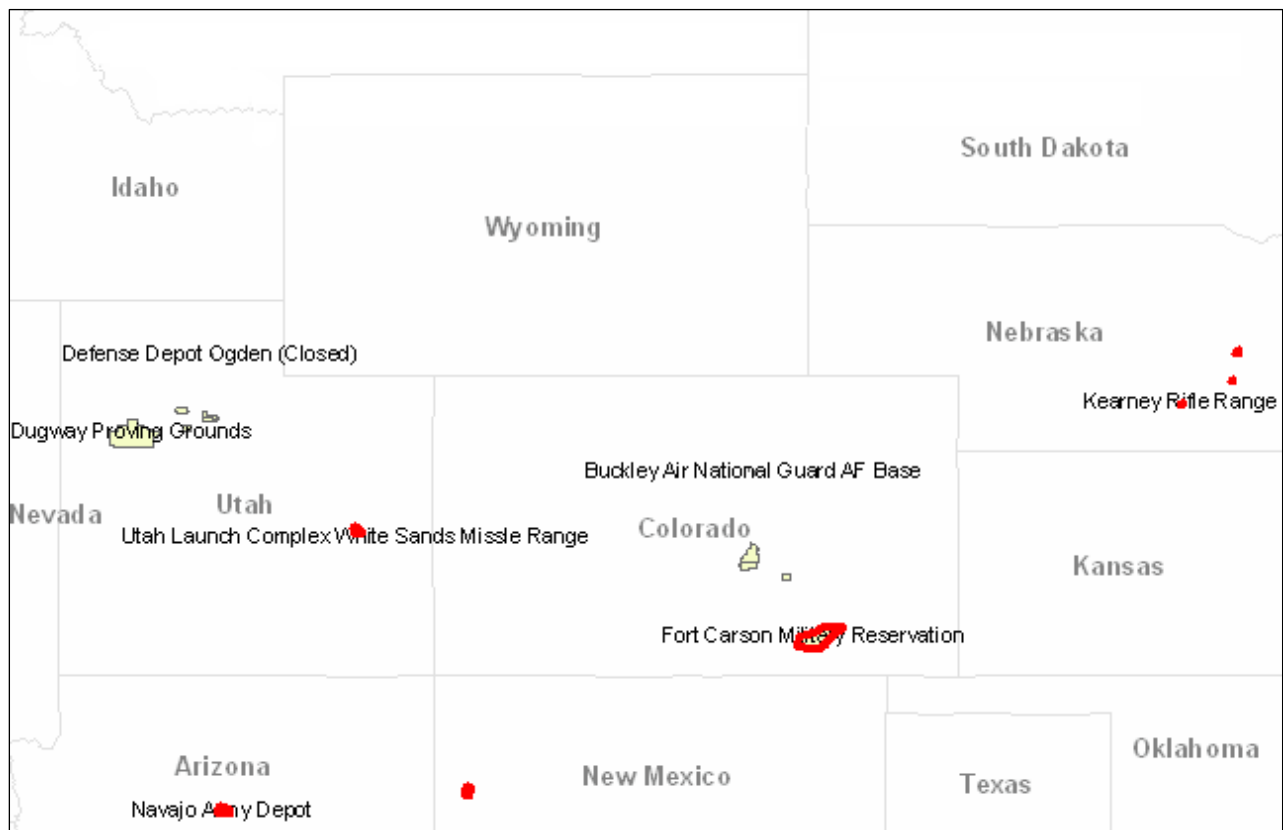


Figure A18. Map illustrating the occurrence of *Sorghastrum nutans* (red outlines) at Army installations in the central and southwestern United States. Data on installations provided in Table A2.

Table A2. Occurrence of selected plant species in counties with co-located Army installations.

Installation Name	State	County	<i>Achnatherum hymenoides</i>	<i>Agropyron smithii</i>	<i>Andropogon gerardii</i>	<i>Bouteloua gracilis</i>	<i>Elymus canadensis</i>	<i>Eragrostis trichoides</i>	<i>Panicum virgatum</i>	<i>Sorghastrum nutans</i>	<i>Achillea millefolium</i>	<i>Amaranthus retroflexus</i>	<i>Asclepias syriaca</i>	<i>Datura stramonium</i>	<i>Ipomoea lacunosa</i>	<i>Portulaca oleracea</i>	<i>Polygonum pensylvanicum</i>	<i>Sida spinosa</i>	<i>Abutilon theophrasti</i>
Anniston Army Depot	Alabama	Calhoun	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fort Rucker	Alabama	Coffee	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fort Rucker	Alabama	Dale	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wheeler National Wildlife Refuge	Alabama	Madison	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Redstone Arsenal	Alabama	Morgan	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fort Benning	Alabama	Russell	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fort Richardson	Alaska	Anchorage	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fort Greely	Alaska	Denali	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fort Wainwright	Alaska	Fairbanks North Star	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fort Greely	Alaska	Southeast Fairbanks	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gulkana Glacier Training Site	Alaska	Valdez-Cordova	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fort Wainwright	Alaska	Yukon-Koyukuk	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fort Huachuca	Arizona	Cochise	0	0	0	1	1	0	0	1	1	0	0	1	0	1	1	1	0
Navajo Army Depot	Arizona	Coconino	1	1	1	1	1	0	1	1	1	1	0	0	0	1	0	0	0
Yuma Proving Ground	Arizona	La Paz	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Buckeye National Guard Target Range	Arizona	Maricopa	0	1	0	1	1	0	0	0	0	0	0	0	0	1	0	0	1
Florence Military Reservation	Arizona	Pinal	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Yuma Proving Ground	Arizona	Yuma	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Camp Joseph T. Robinson	Arkansas	Faulkner	0	0	1	0	1	0	1	1	1	0	0	0	1	1	1	1	1
Fort Chaffee (Closed)	Arkansas	Franklin	0	0	1	0	0	0	1	1	1	0	0	1	1	1	1	1	0
Pine Bluff Arsenal	Arkansas	Jefferson	0	0	1	0	0	0	1	1	1	0	0	1	1	1	1	1	1

Installation Name	State	County	<i>Achnatherum hymenoides</i>	<i>Agropyron smithii</i>	<i>Andropogon gerardii</i>	<i>Bouteloua gracilis</i>	<i>Elymus canadensis</i>	<i>Eragrostis trichoides</i>	<i>Panicum virgatum</i>	<i>Sorghastrum nutans</i>	<i>Achillea millefolium</i>	<i>Amaranthus retroflexus</i>	<i>Asclepias syriaca</i>	<i>Datura stramonium</i>	<i>Ipomoea lacunosa</i>	<i>Portulaca oleracea</i>	<i>Polygonum pensylvanicum</i>	<i>Sida spinosa</i>	<i>Abutilon theophrasti</i>
Fort Carson Military Reservation	Colorado	Las Animas	1	1	1	1	1	1	1	1	1	0	0	0	1	1	0	0	
Fort Carson Military Reservation	Colorado	Otero	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	
Fort Carson Military Reservation	Colorado	Pueblo	1	1	1	1	1	0	0	0	1	0	0	0	0	1	0	0	
Malabar Transmitter Annex	Florida	Brevard	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	
Fort Stewart	Georgia	Bryan	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Hunter Army Airfield	Georgia	Chatham	0	0	0	0	0	0	1	0	1	0	0	1	0	1	1	0	
Fort Benning	Georgia	Chattahoochee	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Fort Gillem Heliport	Georgia	Clayton	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Fort Gordon	Georgia	Columbia	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
Fort Stewart	Georgia	Evans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Fort McPherson	Georgia	Fulton	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	0	
Fort Gordon	Georgia	Jefferson	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Fort Stewart	Georgia	Liberty	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	
Fort Stewart	Georgia	Long	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Fort Benning	Georgia	Marion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Fort Gordon	Georgia	McDuffie	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	
Fort Benning	Georgia	Muscogee	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	
Fort Gordon	Georgia	Richmond	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Fort Benning	Georgia	Talbot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Fort Stewart	Georgia	Tattnall	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Pohakuloa Training Area	Hawaii	Hawaii	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	1	
Schofield Barracks	Hawaii	Honolulu	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	1	
Savanna Army Depot	Illinois	Carroll	0	1	1	0	1	0	1	1	1	0	1	0	0	1	1	0	

Installation Name	State	County	<i>Achnatherum hymenoides</i>	<i>Agropyron smithii</i>	<i>Andropogon gerardii</i>	<i>Bouteloua gracilis</i>	<i>Elymus canadensis</i>	<i>Eragrostis trichoides</i>	<i>Panicum virgatum</i>	<i>Sorghastrum nutans</i>	<i>Achillea millefolium</i>	<i>Amaranthus retroflexus</i>	<i>Asclepias syriaca</i>	<i>Datura stramonium</i>	<i>Ipomoea lacunosa</i>	<i>Portulaca oleracea</i>	<i>Polygonum pensylvanicum</i>	<i>Sida spinosa</i>	<i>Abutilon theophrasti</i>
Aberdeen Proving Ground	Maryland	Baltimore	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Blossom Point Field Test Facility	Maryland	Charles	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
U.S. Garrison, Fort Detrick	Maryland	Frederick	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aberdeen Proving Ground	Maryland	Harford	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Globecom Radio Receiving Station	Maryland	Prince George's	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fort Ritchie	Maryland	Washington	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fort Devens	Massachusetts	Middlesex	0	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1
U.S. Army Reserve Center	Massachusetts	Norfolk	0	0	1	0	1	0	1	1	1	1	1	1	0	1	1	0	1
U.S. Army Reserve Center	Massachusetts	Plymouth	0	0	1	0	0	0	1	1	1	1	1	1	0	1	1	0	0
Fort Devens	Massachusetts	Worcester	0	0	1	0	0	0	1	1	1	1	1	1	0	1	1	0	1
Custer Reserve Forces Training Area	Michigan	Calhoun	0	0	1	0	1	0	1	1	1	0	1	0	0	0	1	0	0
Camp Grayling Military Reservation	Michigan	Crawford	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0
Custer Reserve Forces Training Area	Michigan	Kalamazoo	0	1	1	0	1	0	1	1	1	1	1	1	0	1	1	0	1
Camp Grayling Military Reservation	Michigan	Kalkaska	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
Camp Grayling Military Reservation	Michigan	Missaukee	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Camp Grayling Military Reservation	Michigan	Otsego	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0

Installation Name	State	County	<i>Achnatherum hymenoides</i>	<i>Agropyron smithii</i>	<i>Andropogon gerardii</i>	<i>Bouteloua gracilis</i>	<i>Elymus canadensis</i>	<i>Eragrostis trichoides</i>	<i>Panicum virgatum</i>	<i>Sorghastrum nutans</i>	<i>Achillea millefolium</i>	<i>Amaranthus retroflexus</i>	<i>Asclepias syriaca</i>	<i>Datura stramonium</i>	<i>Ipomoea lacunosa</i>	<i>Portulaca oleracea</i>	<i>Polygonum pensylvanicum</i>	<i>Sida spinosa</i>	<i>Abutilon theophrasti</i>
White Sands Missile Range	New Mexico	Lincoln	1	1	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0
Fort Wingate Depot Activity (Closed)	New Mexico	McKinley	1	1	1	1	1	0	0	1	1	1	0	0	0	1	0	0	0
White Sands Missile Range	New Mexico	Otero	1	1	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0
White Sands Missile Range	New Mexico	Sierra	1	1	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0
White Sands Missile Range	New Mexico	Socorro	1	1	1	1	1	0	0	0	1	1	0	0	0	1	0	0	1
Fort Drum	New York	Jefferson	0	0	1	0	1	0	0	0	0	1	1	0	0	0	1	0	1
Fort Drum	New York	Lewis	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0
Camden Test Annex	New York	Oneida	0	0	1	0	0	0	0	0	1	1	1	1	0	0	1	0	1
West Point U.S. Military Academy	New York	Orange	0	0	1	0	1	0	1	1	1	0	1	1	0	1	1	0	1
Seneca Army Depot (Scheduled to close)	New York	Seneca	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Fort Drum	New York	St. Lawrence	0	0	1	0	0	0	0	0	1	0	1	1	0	1	1	0	1
Military Ocean Terminal Sunny Point	North Carolina	Brunswick	0	0	1	0	0	0	1	1	1	0	0	1	0	1	1	0	0
Fort Bragg Military Reservation	North Carolina	Cumberland	0	0	1	0	0	0	1	1	1	0	0	0	1	0	1	1	0
Fort Bragg Military Reservation	North Carolina	Harnett	0	0	1	0	0	0	1	1	1	0	0	0	0	0	1	0	0
Fort Bragg Military Reservation	North Carolina	Hoke	0	0	1	0	0	0	1	1	1	0	0	0	0	1	1	1	0
Fort Bragg Military Reservation	North Carolina	Moore	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0
Military Ocean Terminal Sunny Point	North Carolina	New Hanover	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0

Installation Name	State	County	<i>Achnatherum hymenoides</i>	<i>Agropyron smithii</i>	<i>Andropogon gerardii</i>	<i>Bouteloua gracilis</i>	<i>Elymus canadensis</i>	<i>Eragrostis trichoides</i>	<i>Panicum virgatum</i>	<i>Sorghastrum nutans</i>	<i>Achillea millefolium</i>	<i>Amaranthus retroflexus</i>	<i>Asclepias syriaca</i>	<i>Datura stramonium</i>	<i>Ipomoea lacunosa</i>	<i>Portulaca oleracea</i>	<i>Polygonum pennsylvanicum</i>	<i>Sida spinosa</i>	<i>Abutilon theophrasti</i>
New Cumberland General Depot	Pennsylvania	York	0	0	1	0	0	0	1	1	1	0	1	1	0	1	1	1	1
Fort Jackson	South Carolina	Kershaw	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	0	1
Fort Jackson	South Carolina	Richland	0	0	1	0	0	0	1	0	1	0	0	1	0	0	1	0	0
Milan Arsenal And Wildlife Management Area	Tennessee	Carroll	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0
Milan Arsenal And Wildlife Management Area	Tennessee	Gibson	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0
Fort Campbell	Tennessee	Montgomery	0	0	1	0	0	0	1	1	1	0	1	1	1	1	1	1	1
Fort Campbell	Tennessee	Stewart	0	0	1	0	0	0	1	1	1	0	1	1	1	1	1	1	1
Camp Swift N. G. Facility	Texas	Bastrop	0	0	1	0	1	0	1	1	1	0	0	0	0	0	1	1	0
Fort Hood	Texas	Bell	0	0	0	1	1	1	1	1	1	1	0	0	1	0	0	0	0
Camp Bullis	Texas	Bexar	0	0	1	1	1	1	0	1	0	1	0	0	0	0	1	0	0
Red River Army Depot	Texas	Bowie	0	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0
Camp Bullis	Texas	Comal	0	0	1	0	0	0	1	1	0	1	0	0	0	0	0	0	0
Fort Hood	Texas	Coryell	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0
Fort Bliss	Texas	El Paso	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Longhorn Ordnance Army Ammo Plant	Texas	Harrison	0	0	1	0	0	0	1	1	0	0	0	0	0	0	1	0	0
Fort Bliss McGregor Range	Texas	Hudspeth	1	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0
Fort Wolters	Texas	Parker	0	0	0	0	1	1	1	1	1	0	0	0	0	0	1	0	0
Utah Launch Complex White Sands Missile Range	Utah	Grand	1	1	0	1	1	0	1	1	1	1	0	0	0	1	0	0	0
Camp Williams	Utah	Salt Lake	1	1	0	0	1	0	0	0	1	1	0	1	0	0	0	0	0
Dugway Proving Grounds	Utah	Tooele	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	1

Installation Name	State	County	<i>Achnatherum hymenoides</i>	<i>Agropyron smithii</i>	<i>Andropogon gerardii</i>	<i>Bouteloua gracilis</i>	<i>Elymus canadensis</i>	<i>Eragrostis trichoides</i>	<i>Panicum virgatum</i>	<i>Sorghastrum nutans</i>	<i>Achillea millefolium</i>	<i>Amaranthus retroflexus</i>	<i>Asclepias syriaca</i>	<i>Datura stramonium</i>	<i>Ipomoea lacunosa</i>	<i>Portulaca oleracea</i>	<i>Polygonum pensylvanicum</i>	<i>Sida spinosa</i>	<i>Abutilon theophrasti</i>
Camp Williams	Utah	Utah	0	0	0	1	1	0	0	0	1	1	0	0	0	0	0	0	1
Defense Depot Ogden (Closed)	Utah	Weber	1	1	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0
Fort Ethan Allen	Vermont	Chittenden	0	0	1	0	1	0	0	1	1	1	1	1	0	1	1	0	1
Arlington National Cemetery	Virginia	Arlington	0	0	0	0	0	0	1	1	1	0	1	1	1	1	1	1	0
Fort Pickett Military Reservation (Closed)	Virginia	Brunswick	0	0	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1
Fort A. P. Hill	Virginia	Caroline	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1
Fort Pickett Military Reservation (Closed)	Virginia	Dinwiddie	0	0	0	0	0	0	1	1	1	0	0	1	1	1	1	1	0
Fort A. P. Hill	Virginia	Essex	0	0	0	0	0	0	1	0	1	0	0	1	1	0	1	1	1
Fort Belvoir	Virginia	Fairfax	0	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1
Warrenton Training Center	Virginia	Fauquier	0	0	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1
Fort Monroe	Virginia	Hampton	0	0	0	0	0	0	1	0	1	0	0	1	1	0	1	0	0
Fort Lee	Virginia	Hopewell	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fort Eustis	Virginia	James City	0	0	0	0	0	0	1	1	1	0	1	1	1	1	1	1	1
Radford Army Ammunition Plant	Virginia	Montgomery	0	0	1	0	0	0	0	1	1	1	0	1	0	0	1	0	1
Fort Eustis	Virginia	Newport News	0	0	0	0	0	0	1	1	1	0	1	1	1	1	1	1	1
Fort Pickett Military Reservation (Closed)	Virginia	Nottoway	0	0	0	0	0	0	0	1	1	0	1	1	1	1	1	1	0
Fort Lee	Virginia	Petersburg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fort Lee	Virginia	Prince George	0	0	1	0	0	0	1	1	1	0	1	1	1	0	1	1	1
Radford Army Ammunition Plant	Virginia	Pulaski	0	0	1	0	0	0	0	1	1	0	1	1	0	0	1	1	1
Fort Story	Virginia	Virginia Beach	0	0	0	0	0	0	1	0	1	0	0	1	1	0	1	0	1

Installation Name	State	County	<i>Achnatherum hymenoides</i>	<i>Agropyron smithii</i>	<i>Andropogon gerardii</i>	<i>Bouteloua gracilis</i>	<i>Elymus canadensis</i>	<i>Eragrostis trichoides</i>	<i>Panicum virgatum</i>	<i>Sorghastrum nutans</i>	<i>Achillea millefolium</i>	<i>Amaranthus retroflexus</i>	<i>Asclepias syriaca</i>	<i>Datura stramonium</i>	<i>Ipomoea lacunosa</i>	<i>Portulaca oleracea</i>	<i>Polygonum pensylvanicum</i>	<i>Sida spinosa</i>	<i>Abutilon theophrasti</i>
Camp Bonneville Military Reservation (Closed)	Washington	Clark	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Nap of the Earth Army Helicopter Training Area	Washington	Kitsap	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Yakama Firing Center	Washington	Kittitas	1	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1
Nap of the Earth Army Helicopter Training Area	Washington	Lewis	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Fort Lewis	Washington	Pierce	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1
Mount Baker Helicopter Training Area	Washington	Skagit	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1
Fort Lewis	Washington	Thurston	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1
Mount Baker Helicopter Training Area	Washington	Whatcom	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1
Yakama Firing Center	Washington	Yakima	1	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	1
Fort McCoy	Wisconsin	Jackson	0	1	1	0	1	0	1	1	1	0	1	0	0	1	1	1	1
Camp Williams	Wisconsin	Juneau	0	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	0
Fort McCoy	Wisconsin	Monroe	0	1	1	0	1	0	1	1	1	0	1	0	0	0	1	1	1
Badger Army Ammunition Plant	Wisconsin	Sauk	0	0	1	1	1	0	1	1	1	1	1	0	0	1	1	1	1

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) (Concluded)

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U.S. Army Engineer Research and Development Center
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14. ABSTRACT (Concluded)

uptake ranged from 8.2 to 437 kg RDX ha⁻¹ in grasses and forbs, respectively. Plant RDX uptake ranged from 3.4 kg ha⁻¹ in grasses to 6.4 kg ha⁻¹ in forbs. Four grasses and one forb metabolized RDX. Two plant species were recommended for further exploration of their phytoextraction/plant-assisted phytoremediation capacity, both species of the uptaker/degrader type. Three other species were recommended for further exploration of their phytostabilization/plant-assisted phytoremediation capacity.