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Candidate Herbaceous Plants for Phytoremediation of Energetics on Ranges

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Abstract: This report identifies rapidly colonizing and resilient grasses/forbs that are tolerant to range-relevant contaminants, with emphasis on TNT and RDX. A literature review identified herbaceous plant species with characteristics that make them potential candidates for use on ranges for phytostabilization and phytoextraction purposes. The 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. Plant species reviewed included 64 grasses and 61 forbs. Based on initial review, eight grasses and eight forbs were selected for tolerance testing. Short-term screening experiments were conducted to evaluate the phytotoxicity of TNT- and RDX-spiked artificial soils to the plants. Seeds were exposed in the laboratory and germination was used as a parameter for plant response. Based on results of this experiment, five grasses and five forbs were identified as rapidly colonizing and short-term tolerant towards TNT- and RDX-contamination of soils.

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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 the ERDC, Construction Engineering Research Laboratory (CERL), Champaign, IL; the University of Illinois; and Analytical Services, Inc., Vicksburg, MS. 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, Research Biologist, Environmental Risk Assessment Branch (ERAB), Environmental Processes and Engineering Division (EPED), EL. Co-principal investigator was Thomas Smith, Wildlife Biologist, Ecological Processes Branch, Installations Division (CN), CERL.

The literature review aimed at identification of rapidly colonizing and resilient herbaceous grass and forb species tolerant towards energetics, reported in Chapter 1, was conducted by Dr. Best and Alan J. Torrey, Staff Scientist, Analytical Services, Vicksburg.

The short-term screening experiments for energetics tolerance through a Petri-dish experiment, reported in Chapter 3, were conducted: (a) for grass species by Dr. Elly Best and Alan Torrey, at ERDC-EL, Vicksburg; and (b) for forb species by Tom Smith, Frank Hagen, ERDC-CERL, and Dr. Jeffrey Dawson, University of Illinois, Urbana-Champaign.

This report was reviewed by Dr. Fiona Crocker, EPED, ERDC-EL, and Dr. Dick Gebhart, CN, ERDC-CERL. Dr. Joan Clarke, EPED, ERDC-EL, advised on the statistics. 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 Richard B. Jenkins 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) because of suspected groundwater contamination by energetics 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-trinitrotoluene (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 have been documented since 1999 (Pennington et al. 2001, 2003, 2004, 2005; Clausen et al. 2004; Efroymson et al. in press) in the United States and in Canada. Contamination pathways 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 the most widely distributed, and both compounds are often found at the same site in the soil. 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 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. (2006), 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*; Cataldo et al. 1989). More recently other phytotoxic concentrations have been reported 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 > 1540 mg RDX kg^{-1} soil failed to reduce the biomass of perennial ryegrass (*Lolium perenne*) and alfalfa (*Medicago sativa*) by 20% 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.

Objectives

The objectives of the current study were to identify rapidly colonizing and resilient plant species (grasses and forbs) that are tolerant towards range-relevant contaminants, with emphasis on TNT and RDX.

2 Literature Review: Identification of Rapidly Colonizing and Resilient Herbaceous Grass and Forb Species Tolerant Towards Energetics

This review identifies herbaceous plant species with characteristics that make them potential candidates for use on ranges for phytostabilization and phytoextraction purposes. The review is limited to native grass and forb species, introduced non-invasive and invasive species, and species with improved genetic characteristics that have successfully been used on training lands in North America and Canada (more than 100 species; Johnson and Biondini 2001; Craine et al. 2002; Palazzo et al. 2003).

Plant characteristics included in the review

Potentially suitable grass and forb species were reviewed for tolerance towards energetics and other characteristics considered important for persistence on military ranges. Tolerance towards RDX is emphasized in the current study. However, tolerance towards TNT and HMX are also included, because TNT is an important determinant of the distribution of plants in energetics-contaminated soils. Compared with RDX and HMX, TNT is the energetic most toxic to plants (Burken 2003; Best et al. 2005). HMX is an energetic commonly present at considerable levels at Canadian Force bases.

Resilience, another important characteristic, was considered also. Resilience is defined as the ability of a vegetative system to recover after disturbance and return to its original state (Doe et al. 1999). Military training exercises often destroy the vegetation and disturb the soil horizon, which leads to soil erosion (Halvorson et al. 2001), increased runoff, and leaching of energetics. The resilience of plants encompasses several traits, the combination of which is usually species-specific. Some plants recover from disturbance by a combination of a short life cycle and high seed production, while others do so by a combination of long life cycle, large root system and considerable regrowth potential. Plant traits describing the life cycle (annual, biennial, and perennial), root system, and shoot system have been included in the review to enable evaluation of resilience. Certain combinations of plant traits may improve viability in the native environment, facilitate introduction into new environments, and even foster dominance to the point that non-native plants may be considered as invasive species. Minimal side effects on the local biotic communities are an objective when using plants in phytoremediation applications, so the preferred candidates would be native plant species, followed by introduced ones, while invasives would have to be avoided. A major objective of phytoremediation is to minimize side effects on local biotic communities. Therefore, native plant species are preferred candidates, followed by introduced plants to the exclusion of invasive species.

Besides being an important trait for resilience, the root system greatly affects the successful use of a given plant species for phytoremediation purposes. The rooting depth of herbaceous plants is usually limited to the 12 to 25 cm of top soil. However, plant roots respond to varying conditions often found in the horizons of a soil profile. Roots tend to proliferate in the A-horizon because it is less compacted, better aerated, and more fertile than in the horizons below. Improvement of soil fertility in the A-horizon not only enhances root growth there, but may increase the vigor and extent of the root system deeper in the profile as well. Accessibility of soil contaminants to plants is a function of rooting depth, with tap roots usually penetrating deeper into the soil profile than fibrous roots. The degree of dissection, spatial distribution in the soil and total mass of the root system determines the extent of contact between contaminants and plants, with frequently dissected, high mass, fibrous roots having the greatest contact potential.

The photosynthetic pathway is an additional characteristic important for plant survival with the C_4-pathway providing a larger survival capacity at low water availability than the C_3-pathway. Ranges are often located in arid areas. This characteristic is included in the description of the species selected for the screening experiments described in Chapter 3, but it has not been included for all species in the literature review.

Energetics-tolerant, resilient plant species have been listed in relation to soil contaminant concentrations and mixtures in recent reports on range contamination (Pennington et al. 2001, 2003, 2004, 2005; USACE-AK 2001). The presence of these plants confirms their ability to persist on ranges, which may make them suitable candidates for phytoremediation purposes. These plant species have been included in the review, and the sites where they were found have been documented. Plant species have typical biogeographic distribution patterns (i.e., that they only grow in certain areas of the country: southern species do not grow in the North and vice versa).

This review focuses on the following regions of the United States: East, Mid-West, North-West, North, South, South-West, West, and West-Central. Additionally, U.S. states and Canadian provinces with confirmed occurrences of these plant species have been documented. The biogeographical distribution of the plant species is an important characteristic that strongly determines the plants' application potential for phytoremediation purposes.

Resistance to selected metals (such as copper and cadmium) that originate from projectile casings and are also toxic to plants was not included in the review, since these metals are usually present at very low bioavailable levels.

Results and discussion

All herbaceous plant species identified in the literature are listed with their characteristics in Tables 1 and 2.

Table 3 shows that, among the 64 grass species, 35 were native and 29 introduced (4 of these being invasive). Twenty-two grass species (37.5% of the grasses) had been screened for tolerance towards one or more energetics, but no native species had been screened for tolerance towards TNT and RDX. Table 3 also shows that, among the 61 forb species, 29 were native and 32 introduced (3 of these being invasive). Thirty-two (60.6% of the forbs) had been screened for tolerance towards one or more energetics, and one native species (sunflower, *Helianthus annuus*) had been screened for tolerance towards TNT and RDX.

From the reviewed plant species, eight grass and eight forb species were selected for inclusion in the short-term screening experiments for TNT and RDX tolerance (Table 4). This selection was based on:

- All criteria included in the review (i.e., tolerance towards energetics, resilience-related life cycle characteristics and plant traits, typical biogeographic distribution, including documented occurrence on Army installations).

Table 1. Characteristics of phytoremediation candidate grass species.

Species name	Common Name	Screened[1]	Recon[2]	Life Cycle[3]	Root Size, Type	Shoot Size	Region US[4]	State US, Prov. CA[5]	Site	Ref.
					Native Grasses					
Achnatherum hymenoides	Indian ricegrass			P	large	small	W-C	CO	Ft. Carson	1,2
Agropyron Smithii	Western wheatgrass	HMX	yes	P	large	small	N&S	Alberta, AK	CFB	3,4
Agropyron spicatum	Bluebunch wheatgrass			P	small	medium	W	WA, AK	Yakima	5
Agropyron subsecundum	Wheatgrass		yes	P	small	medium	N&S	AK	Ft. Greely	6
Andropogon Gerardii	Big bluestem	TNT	yes	P	very large	very large	N&S	NE	NOP	7
Bouteloua curtipendula	Sideoats grama			P	small	medium	N&S	CO	Ft. Carson	1,2
Bouteloua gracilis	Blue grama			P	large	small	N&S	CO	Ft. Carson	
Bromus sitchensis	Brome grass	HMX	yes	P	small	small	N	Alberta	CFB	3
Bromus sp.	Brome	TNT, HMX		P			N&S	AK, HW		4
Calamagr. purpurascens	Reedgrass		yes	P	very small	medium	N-W	AK	Ft. Greely	6
Carex supine	Sedge		yes	P			N	AK	Ft. Greely	6
Elymus arenarius	Beachrye		yes	P	large	large	N&S	AK	Ft. Greely	6
Elymus canadensis	Canadian wild rye	TNT		P	large	medium	N&S			8
Elymus lanceolatus	Thickspike wheatgrass			P	large	small	N-W	AK, CO	Ft. Carson, Yakima	1, 2, 9
Elymus tachycaulus	Slender wheatgrass			P	large	medium	N-W	AK, CO	Ft. Carson, Pikes Peak	1, 2, 5
Elymus wawawaiensis	Snake River wheatgrass			P	large	medium	N-W	WA	Yakima	1, 5, 9
Eragrostis trichoides	Sand lovegrass			P	large	large	MW	CO	Ft. Carson	2
Festuca ovina var.	Hard fescue			P	medium	small	W	NH	G.c. CCREL	10
Festuca rubra L.	Red fescue	TNT	yes	P	small	small	N&S	AK, HW, NH	Ft. Greely, g.c.	4, 6, 10

Species name	Common Name	Screened[1]	Recon[2]	Life Cycle[3]	Root Size, Type	Shoot Size	Region US[4]	State US, Prov. CA[5]	Site	Ref.
									CCREL	
Festuca sp.	Fine fescues			P			E	NY	Ft. Drum	1
Goldar whitnar	Bluebunch wheat-grass			P	small	medium	N-W			11
Koeleria gracilis	Koeleria	HMX	yes	P	small	very large	N&S	Alberta, HW	CFB	3
Leymus cinereus	Basin wildrye			P	large	very large	W	WA	Yakima	1, 5
Leymus triticoides	Beardless wheatgr.			P	small	medium	W			1
Nassella pulchra	Purple needlegrass			P	small	medium	W	CA, CO	Ft. Carson	1
Panicum virgatum	Switchgrass	TNT		P	very large	medium	N&S	HW, NE, NY	Ft. Drum, NOP	1, 4, 7
Pascopyrum smithii	Western wheat-grass			P	large	small	N&S	AK, CO	Ft. Carson	1, 2, 9, 5
Poa glauca	Glaucous blue-grass		yes	P	small	small	N-W	AK	Ft. Greely	6
Poa pratensis	Kentucky blue-grass	RDX		P	small	small	N&S			1, 20
Poa sandbergii	Sandberg blue-grass			P	small	small	W	WA	Yakima	1, 2, 5
Pseudoroegneria spicata	Bluebunch wheat-grass			P	small	medium	W	AK, WA	Yakima	1, 9
Schizachyrium scoparium	Little bluestem			P	medium	medium	N&S	NY	Ft. Drum	1
Sorghastrum nutans	Indian grass		yes	P	very large	large	N&S	NE	NOP	7
Sporobolus airoides	Alkali sacaton			P	large	medium	W	CO, HW, PR	Ft. Carson	2
Sporobolus cryptan-drus	Sand dropseed			P	large	medium	N&S	CO	Ft. Carson	2

Species Name	Common Name	Screened[1]	Recon[2]	Life Cycle[3]	Root Size, Type	Shoot Size	Region US[4]	State US, Prov. CA[5]	Site	Ref.
					Introduced Grasses					
Aira L.	Hairgrass			A	small	small	N&S	AK, HW, NY	Ft. Drum	1
Bromus inermis	Smooth brome-grass		yes	P	medium	large	N&S	AK, IL, NE	JAAP, NOP	7, 12
Cyperus esculentus	Yellow nutsedge	TNT, RDX	yes	P	small	small	N&S	AK, HW, NE, PR, VI	NOP	4, 7, 12, 13
Agropyron cristatum	Crested wheat-grass			P	large	small	N&S	AK, CO	Ft. Carson	1, 5, 9
Agropyron desertorum	Desert crested wheatgrass			P	large	small	N&S	AK, CO	Ft. Carson	1, 9
Agropyron fragile	Siberian wheat-grass			P	small	medium	W	CO	Ft. Carson	1, 2, 9
Agrostis gigantea	Bentgrass or red-top		yes	P	very large	small	N&S	AK	Ft. Greely	6
Alopecurus pratensis	Meadow foxtail	TNT		P	medium	medium	N&S			4
Avena sativa	Oat	TNT	yes	A	small	small	N&S	AK, HW, IL, PR	JAAP	12
Bromus mollis	Blando brome	TNT		A	small	medium	N&S	AK, HW	JAAP	12
*Bromus tectorum**	Cheatgrass			A	small	medium	N&S	AK, HW		1
Dactylis glomerata	Orchardgrass	RDX		P	medium	medium	N&S			20
Eragrostis curvula	Weeping lovegrass			P	medium	medium	N&S	HW, PR		1
Festuca arundinacea	Tall fescue	TNT	yes	P	large	large	N&S	AK, HW, NE, NH	NOP, G.c. CCREL	7, 10, 20
Festuca brevipila	Hard fescue			P	small	small	N&S			1
Festuca ovina L.	Sheep fescue			P	small	very small	N-W	NH	G.c. CCREL	1, 10
Festuca rubra var.	Chewings fescue			P	medium	medium	N-W	NE, NH	G.c. CCREL	10
Hordeum sativum	Barley	TNT		A	medium	medium	N&S	AK, HW		4
Lolium multiflorum	Ryegrass	TNT		AP	small	medium	N&S			4
Lolium perenne	Perennial ryegrass	TNT,RDX HMX	yes	AP	small	medium	N&S	IL	JAAP	4, 12, 20

Species Name	Common Name	Screened[1]	Recon[2]	Life Cycle[3]	Root Size, Type	Shoot Size	Region US[4]	State US, Prov. CA[5]	Site	Ref.
Phleum sp.	Timothy	TNT		P	small	medium	N&S	AK, HW		4
Psathyrostachys juncea	Russian wildrye			P	large	medium	N&S	CO	Ft. Carson	2, 5, 9
Sorghum bicolorx S.sudan.	Sorghum x sudangr.	TNT		A	medium	medium	N&S			12
*Sorghum halepense**	Johnsongrass	TNT		P	small	medium	N&S			8
Sorghum sudanese	Sorghum	RDX		A	medium	medium	N&S			4, 14
*Taeniatherum asperum**	Medusahead rye			A	medium	large	W	NE		1
Thinopyrium interm. barb.	Intermediate wheatgrass			P	large	medium				9
*Triticum aestivum**	Wheat	TNT, RDX , HMX		A	large	medium	N&S			4, 12, 13, 14, 20
Zea mays	Corn	TNT, RDX		A	small	large	N&S			4, 12, 13, 20

[1] Screened for energetics tolerance; [2] Found at contaminated site during reconnaissance; [3] A, annual; B, biennial; P, perennial;

[4] E, East; M-W, Mid-West; N, North; N-W, North-West; N&S, North and South; S-W, South-West; W, West;

[5] CFB, Canadian Forces Base; JAAP, Joliet Army Ammunition Plant; LAAP, Louisiana Ammunition Plant; NOP, Nebraska Ordnance Plant;

* Considered as invasive in at least one U.S. state.

For references see Table 2.

Table 2. Characteristics of phytoremediation candidate herbaceous forb species.

Species Name	Common Name	Screened[1]	Recon[2]	Life Cycle[3]	Root Size, Type	Shoot Size	Region US[4]	State US, Prov. CA[5]	Site	Ref.
Native Forbs										
Achillea millefolium	Common yarrow			P	small	small	N&S	AK, WA	Yakima	1, 5, 15
Amaranthus retro-flexus	Redroot pigweed			A	medium	medium	N&S			16
Anemone multifida	Pacific anemone	HMX	yes	P	very small	small	N-W	Alberta	CFB	3, 4
Artemisia gna-phalodes	Western sage	HMX	yes	P	large	medium	N&S	Alberta	CFB	3, 4
Artemisia tridentata	Big sagebrush			P	very large	large	W	AK		1
Asclepias syriaca	Common milkweed		yes	P	tap	medium	N	IL	JAAP	12
Aster sibiricus	Siberian aster		yes	P	small	small	W	AK	Ft. Greely	6
Astragalus drum-mondii	Drummond's milk vetch	HMX	yes	P	large	medium	N-W	Alberta	CFB	3
Astralagus alpinus	Standing milk vetch		yes	P	small	small	N-W	AK	Ft. Greely	6
Draba sp.	Rock cress		yes	AB	small	small	N&S	AK	Ft. Greely	6
Helianthus annuus	Sunflower	TNT, RDX		A	small	very large	N&S	AK, HW, PR		4, 13, 20
Ipomoea lacunosa	Morning glory			A	small	medium	N&S			16
Kuhnia eupatorioides	False boneset		yes	P	tap	small	S	IL	JAAP	12
Lespedeza capitata	Bushgrass	RDX		P	medium	medium	E			20
Mentha sp.	Mint	RDX		P	very small	medium	N&S			17
Minuartia sp.	Sandwort		yes	AP	medium	medium	N-W	AK	Ft. Greely	6
Monarda fistulosa	Wild bergamot	HMX	yes	P	small	medium	N&S	Alberta	CFB	3, 4
Oxytropis campestris	Field oxytrope		yes	P	small	small	N-W	AK	Ft. Greely	6
Phacelia sericea	Bluebell	TNT		P	tap	small	W	AK		4
Physalis heterophylla	Clammy ground cherry		yes	P	small	medium	N&S	IL	JAAP	12
Polygonum pensylv.	Pensylv. smartweed			A	small	medium	S-W			16
Portulaca oleracea	Common purslane			A	small	small	N&S			16
Senecio sp.	Groundsel		yes	P	medium	medium	E	AK	Ft. Greely	6
Sida spinosa	Prickly sida			A	small	medium	N&S			16

Species Name	Common Name	Screened[1]	Recon[2]	Life Cycle[3]	Root Size, Type	Shoot Size	Region US[4]	State US, Prov. CA[5]	Site	Ref.
Solidago decumbens	Goldenrod	RDX,HMX	yes	P	small	medium	N&S	AK, Alberta	Ft. Greely	18
Stellaria monantha	Common chickweed		yes	P	small	small	N-W	AK	Ft. Greely	6
Symphoricarpos albus	Common snowberry	HMX	yes	P	small	medium	N-W	AK, Alberta	CFB	3
Vaccinium sp.	Blueberry	HMX	yes	P	medium	large	N&S	Alberta	CFB	3, 4
Verbena hastata	Blue vervain		yes	P	small	medium	N&S	IL	JAAP	12
Introduced Forbs										
Abutilon avicennae	Velvet leaf	TNT		A	small	medium	N&S			8
Allium schoenoprasum	Wild chives	TNT		P	small	small	N	AK		4
Brassica rapa	Canola	RDX,HMX		AB	medium	medium	N&S	AK, HW, IL, PR, VI		4, 20
Bupleurum triradiatum	Thorough-wax		yes	P	small	small	N-W	AK	Ft. Greely	6
Catharanthus roseus	Periwinkle	TNT,RDX, HMX		AP	small	small	S	HW, PR, VI		4, 13, 14
Cicer arietinum	Chickpea	TNT		A	large	very large	N&S	PR		4
Cichorium intybus	Chicory		yes	BP	small tap	large	N&S	IL	JAAP	12
*Circium vulgare**	Common thistle	HMX	yes	B	medium	large	N&S	AK, Alberta, HW	CFB	3,4
Coleus sp.	Coleus	RDX		A	small	medium	N&S			17
Cucumis sativa	Cucumber	RDX		A	small	large	E	HW, PR		4, 20
Datura innoxia	Jimson weed.prickly burr	TNT		AP	small	medium	W	HW, PR, VI		8, 20
*Datura stramonium**	Jimson weed	TNT		A	small	medium	N&S		LAAP	19
Daucus carota	Queen Anne's lace	RDX	yes	B	tap	medium	N&S	IL, PR	JAAP	12, 13
Dipsacus sylvestris	Common teasel		yes	B	tap	large	N&S	IL	JAAP	12
Glycine max	Soybean	TNT,RDX		A	small	medium	E	PR		4, 13, 14
Hibiscus cannabinus	Kenaf	TNT		A	small	medium	S	FL, PR		4
Lactuca sativa	Lettuce	RDX		AP	small	medium	N&S	PR, VI		4, 13, 14, 20

Species Name	Common Name	Screened[1]	Recon[2]	Life Cycle[3]	Root Size, Type	Shoot Size	Region US[4]	State US, Prov. CA[5]	Site	Ref.
Linum perenne	Wild flax		yes	P	small	small	N	AK	Ft. Greely	6
Lupinus angustifolius	Lupin	TNT		A	small	large	E	FL, NY		4
Lycopersicum esculentum	Tomato	TNT, RDX		A	small	medium	N&S			4, 13, 14
Lycopersicon peruvianum	Tomato	TNT		A	small	medium	N&S			8
Medicago sativa	Alfalfa	TNT, RDX, HMX		AP	very large	medium	N&S	AK, HW, IL, PR, VI	JAAP	1, 4, 13, 12
M. sativa Ladak	Ladak alfalfa			P	very large	medium	N&S	CO	Ft. Carson	2
Onobrychis viciifolia	Sanfroin	RDX		P	large	large	N&S			20
Phaseolus vulgaris	Bush bean	TNT, TNT, HMX		A	medium	medium	N&S	IL, PR, VI	JAAP	4, 12.,13
Pisum sativum	Pea	TNT		A	small	large	N&S			4
Raphanus sativus	Radish	RDX		A	tap	small	N&S			13, 18
Sanguisorba minor	Delar small burnet	RDX		P	medium, tap	medium	E&W			20
Spinacia oleracea	Spinach	RDX		A	small	small	N&S	AK		13
Taraxacum sp.	Dandelion		yes	P	very small	small	N&S	AK	Ft. Greely	6
Trifolium pretense	Red clover	RDX		P	medium	medium	N&S			20
*Trifolium repens**	White clover	TNT, RDX	yes	BP	medium	small	N&S	NE	NOP	4,7, 20

[1] Screened for energetics tolerance; [2] Found at contaminated site during reconnaissance; [3] A, annual; B, biennial; P, perennial;

[4] E, East; M-W, Mid-West; N, North; N-W, North-West; N&S, North and South; S-W, South-West; W, West;

[5] CFB, Canadian Force Base; JAAP, Joliet Army Ammunition Plant; LAAP, Louisiana Ammunition Plant; NOP, Nebraska Ordnance Plant;

* Considered invasive in at least one U.S. state.

References: 1.Palazzo et al. 2003; 2. Waldron et al. 2006a; 3. Groom et al. 2002; 4. Best et al. 2005; 5. Hardy and Palazzo 2002; 6. USACE-AK 2001; 7. Krishnan et al. 2000; 8. Ouyang et al. 2005; 9. Asay et al. 2001; 10. Palazzo and Brar 1997; 11. Larson et al. 2000; 12. Zellmer et al. 1995; 13. Major et al. 2002; 14. Burken 2003; 15. Waldron et al. 2006b; 16. Hagen, F.L., Construction Engineering Research Laboratory, Champaign, IL, pers. comm. 2006; 17. Reynolds et al. 2006; 18. Price et al. 2002; 19. Lucero et al. 1999; 20. Winfield et al. 2004.

Table 3. Total number of herbaceous plant species reviewed. The number of plants screened for explosives tolerance is indicated between parentheses.

Category	Herbaceous Plant Species	
	Grasses	Forbs
Native	35 (9)	29 (11)
Introduced	25 (13)	29 (23)
Invasive	4 (2)	3 (3)
Total	64 (24)	61 (37)

- Seed size. Plant species with extremely small seeds were not used for the experiments, because it proved to be impossible to verify germination by the appearance of roots or shoots using a light microscope.
- Availability of propagules. The availability of propagules of grass and forb species considered for inclusion in the short-term screening experiments was explored by contacting various commercial seed vendors. In addition, the availability of natural and inbred, SERDP-selected, germplasms of grass species considered as candidates was verified by contacting A. J. Palazzo at the Cold Regions Research and Engineering Laboratory (ERDC-CRREL), Hanover, NH and M. Biondini at the Department of Animal and Range Sciences, North Dakota State Univ., Fargo.
- Photosynthetic pathway. Plants with a C_3 pathway as well as plants with a C_4 pathway were included in the selection.
- Exceptional traits. Possession of a prostate growth form affecting large soil surfaces to a shallow depth.
    - *Polygonum pensylvanicum*, which reproduces largely vegetatively by tubers.
    - *Portulaca oleracea*
- Other criteria.
    - *Achillea millefolium* has not been screened for TNT tolerance, but unpublished information indicated that the related *A. millefolium* var. occidentalis (Western yarrow) has been screened and may be tolerant.
    - *Datura stramonium* has been suggested to metabolize TNT from TNT-contaminated process ('pink') water lagoons. It is considered an invasive weed in Nebraska and several southern states, but is not federally listed as a noxious weed.
    - *Ipomoea lacunosa* L. was selected as a substitute for *Astragalus drummondii*, since seeds of the latter plant were not available.

Table 4. Herbaceous plant species selected for short-term tolerance screening towards TNT and RDX.

Species Name	Common Name	Family	Screened[1]	Life Cycle[2]	Root Size, Type	Shoot Size	Seed Quantity	Seed Size	Photosynthetic Pathway	Region US[3]
Grasses										
Achnatherum hymenoides	Indian ricegrass	Poaceae		P	large	small	many	considerable	C_3	W-C
Agropyron Smithii	Western wheatgrass	Poaceae	HMX	P	large	small	many	considerable	C_3	N&S
Andropogon Gerardii	Big bluestem	Poaceae	TNT	P	very large	very large	many	considerable	C_4	N&S
Bouteloua gracilis	Blue grama	Poaceae		P	large	small	many	small	C_4	N&S
Elymus canadensis	Canadian wild rye	Poaceae	TNT	P	large	medium	many	considerable	C_3	N&S
Eragrostis trichoides	Sand lovegrass	Poaceae		P	large	large	many	very small	C_4	MW
Panicum virgatum	Switchgrass	Poaceae	TNT	P	very large	medium	many	considerable	C_4	N&S
Sorghastrum nutans	Indiangrass	Poaceae		P	very large	large	many	considerable	C_4	N&S
Forbs										
Achillea millefolium	Western yarrow	Asteraceae		P	small	small	many	very small	C_3	N&S
Amaranthus retroflexus	Redroot pigweed	Amaranthaceae		A	medium	medium	many	large	C_4	N&S
Asclepias syriaca	Common milkweed	Asclepiadaceae		P	tap	medium	considerable	large	C_4	N
Datura stramonium[4]	Jimson weed	Solanaceae	TNT	A	small	medium	considerable	considerable	C_3	N&S
Ipomoea lacunosa	Morning glory	Convolvulaceae		A	small	medium	considerable	considerable	C_3	N&S
Polygonum pensylvanicum	Pensylv. smartweed	Polygonaceae		A	small	medium	5	5	C_3	S-W
Portulaca oleracea	Common purslane	Portulacaceae		A	small	small	many	small	C_4, CAM	N&S
Sida spinosa	Prickly sida	Malvaceae		A	small	medium	many	considerable	C_3	N&S

[1] Screened for explosives tolerance.
[2] A, annual; P, perennial.
[3] E, East; M-W, Mid-West; N, North; N-W, North-West; N&S, North and South; S-W, South-West; W, West;.
[4] Introduced species.
[5] Reproduction largely vegetativey by tubers.

Conclusions

A literature review was conducted to identify herbaceous plant species with characteristics that make them potential candidates for use on ranges for phytostabilization and phytoextraction purposes. Eight criteria were used on which to base the selection of plant species for inclusion in short-term screening experiments for tolerance towards TNT and RDX. These criteria were:

1. tolerance towards energetics,
2. resilience-related life cycle characteristics and plant traits,
3. typical biogeographic distribution (including documented occurrence on Army installations),
4. seed size,
5. availability of propagules,
6. photosynthetic pathway,
7. exceptional traits, and
8. other.

A total of 125 herbaceous plant species was reviewed: 64 grasses and 61 forbs. Based on the initial review, eight grasses and eight forbs were selected, to include:

1. The grasses —
   a. *Achnatherum hymenoides*,
   b. *Agropyron Smithii*,
   c. *Andropogon Gerardii*,
   d. *Bouteloua gracilis*,
   e. *Elymus canadensis*,
   f. *Eragrostis trichoides*,
   g. *Panicum virgatum*, and
   h. *Sorghastrum nutans*;
2. The forbs —
   a. *Achillea millefolium*,
   b. *Amaranthus retroflexus*,
   c. *Asclepias syriaca*,
   d. *Datura stramonium*,
   e. *Ipomoea lacunosa*,
   f. *Polygonum pensylvanicum*,
   g. *Portulaca oleracea*, and
   h. *Sida spinosa*.

3 Short-Term Screening for Energetics Tolerance

Introduction

Short-term screening experiments for tolerance towards TNT and RDX were conducted, following standard testing procedures, based on the Standard Guide for Conducting Plant Toxicity Tests, category 'Short-term, physiological endpoints' (ASTM 1999) and modified by Best et al. (2004, 2006). In these experiments eight grass and eight forb species (identified in Chapter 2) were evaluated. Short-term survival and presence of a well-developed root system were used as measures of tolerance. The latter characteristic is particularly important for phytostabilization purposes. Plants exposed to high RDX-concentrations may form substantial above-ground biomass, but minimize exposure of their root system to RDX (as reported by Best et al. 2006 in perennial ryegrass and alfalfa), making them unsuitable for phytostabilization/phytoextraction on ranges.

Material and methods

Experimental

Short-term screening experiments were conducted to evaluate the phytotoxicity of artificial soil, spiked with, respectively, TNT and RDX for the selected plants. Dose-response curves for TNT between 0 and 100 mg kg^{-1} soil dry weight (DW) and for RDX between 0 and 1,000 mg kg^{-1} DW were constructed for the tests. TNT and RDX at 100 and 1000 mg kg^{-1} DW, respectively, were considered high enough to cause significant effects on the germination of the seeds, based on similar tests with other plant species (Best et al. 2004, 2006).

An artificial soil, prepared according to the Organization for Economic Cooperation and Development (OECD) method (OECD 1984), was used as a substrate, because it is well-characterized, widely used in toxicity testing, and suitable to support seed germination. This soil is composed of 70% (w/w) grade No 4 sand (Ash Grove, Jackson, MS), 20% of colloidal kaolinite clay (Carolina Biological, Burlington, NC), and 10% 2-mm *Sphagnum* peat (milled horticultural *Sphagnum* moss, Mosser Lee Company, Millston, WI).

The test soils were prepared by spiking with different volumes from the same methanolic stock solution. Solvent-spiked soil served as a reference. Water-spiked soil served as a test to verify plant performance. All treatments were replicated seven times. Treatments for grasses followed a randomized block design, in two blocks. Treatments for forbs followed a completely randomized design. The studies on the grasses and forbs each included a total of 560 test units:

- for the TNT-tests per grass and forb group (1 reference x 8 species x 7 replicates) + (3 TNT treatments x 8 species x 7 replicates) + (1 water control x 8 species x 7 replicates)
- for the RDX-tests per grass and forb group (1 reference x 8 species x 7 replicates) + (3 RDX treatments x 8 species x 7 replicates) + (1 water control x 8 species x 7 replicates)

For the TNT tests, artificial soils were spiked with 10-, 50-, and 100-mg TNT kg^{-1} DW using methanol as a solvent, and they were amended with reverse osmosis (RO) water up to a total volume of 5.5 mL. For the RDX tests, artificial soils were spiked with 100-, 500-, and 1,000-mg RDX kg^{-1} DW. After spiking, the soils were mixed with a stainless-steel spatula, and placed in a vented fume hood without illumination overnight to allow the methanol to evaporate prior to exposure of the test organisms.

The parameter used to measure plant response was seed germination, observed as root emergence visible under a light microscope at the end of the cultivation period. All test units were inspected every other day, and the emergence of roots and shoots was recorded. Based on experience with the grass *Lolium perenne* and the forb *Medicago sativa* (Best et al. 2004, 2006), a cultivation period of 13-14 days was considered long enough to observe phytotoxicity. This observation period proved appropriate for the grasses. Germination in the forbs was followed over a period of up to 34 days, because it was less synchronous and appeared to be slower than in the grasses. By inspection of all recorded data, it was found that almost all forbs (except those that did not germinate at all) exhibited roots earlier than the grasses and for a few days only, which were resorbed subsequently for shoot formation. Therefore, an observation period of 4 to 12 days was used for the forbs, and the maximum number of roots that emerged in this period was used as the parameter for plant response.

Plant materials

Propagules of the plant species identified as candidates for the screening experiment, previously discussed and listed in Table 4, were obtained as follows.

All grass seeds were purchased from the Granite Seed Company, Lehi, UT.

The forbs were purchased from five vendors:

- *A. retroflexus*, *I. lacunosa*, and *S. spinosa* from Azlin Seed Service, Leland, MS
- *D. stramonium* and *P. pensylvanicum* from the University of Illinois Department of Crop Science Seed Inventory, Urbana, IL
- *A. millefolium* from Easywildflowers, Willow Springs, MO
- *A. syriaca* from Prairiemoon Nursery, Winona, WI
- *P. oleracea* from Monsanto Seed Library, St. Louis, MO

Plant exposures

For each unit, 25 seeds were freed from chaff, counted, and placed on top of 5 g of the appropriate soil mixture contained in 15-mL Petri dishes. Plants were cultivated as follows: (1) the grasses in a walk-in growth chamber of the Environmental Laboratory, Vicksburg, MS, and (2) the forbs in two growth cabinets of the University of Illinois, Urbana-Champaign, Champaign, IL, illuminated with 500-600 $\mu E\ m^{-2}\ s^{-1}$ at the seed surface at a 16-h photoperiod and temperature of 22-26 ^oC. For grasses, the TNT-test lasted from 15 to 29 June (14 days), and the RDX-test from 13 to 26 July 2006 (13 days). For forbs, the TNT-test lasted from 8 November to 8 December 2006 (30 days), and the RDX-test from 13 January to 16 February 2007 (34 days). The Petri dishes were sprayed with RO water immediately after placing the seeds on the soils, and, subsequently, every day as needed. A moisture level at field capacity allows maximum specific mass transport of contaminants with soil solution, and direct contact of contaminant and seed. The seeds were harvested after 13 to 34 days of cultivation.

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 high performance liquid chromatography (HPLC) analysis indicated 1% trinitrobenzene (TNB). The technical RDX was purified by two successive recrystallization cycles in water at 100 °C. Verification of RDX using HPLC analysis indicated 4% HMX. The purities were considered appropriate for metabolic studies. Energetics standards were purchased from Accu Standard Inc., Ellington, CT.

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. We transformed the counted numbers of seeds that germinated out of the original 25 seeds to percentage germinated seeds prior to analysis of variance (ANOVA). ANOVA was expanded 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 level (p value of ≤ 0.05).

Results and discussion

Grasses

TNT exposures

Germination of the grass seeds included in the TNT exposure experiment was significantly affected by TNT concentration ($p=0.025$), species ($p<0.001$), and by their interaction ($p=0.006$; Table 5). The block ($p=0.146$) effects were not significant; therefore, all data were statistically analyzed as if completely randomized. The solvent effect was also not significant ($p=0.714$); therefore, both the methanol-spiked references and the water controls were included in the dataset for further analysis. 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 1, germination greatly differed with species, being very poor in *A. hymenoides* (AH), low in *A. Smithii* (AS) and *P. virgatum* (PV), intermediate in *A. Gerardii* (AG), *E. canadensis* (EC), and *S. nutans* (SN), and significantly higher than in all other grasses in *B. gracilis* (BG) and *E. trichoides* (ET). The only species in which germination was significantly affected by

Table 5. Germination of candidate grass species in response to 14 days of exposure to TNT-contaminated soil. Mean values and standard deviations are shown (N=7). ANOVA results[1] are listed.

TNT exposure grasses			
Species	Germination (%)		
A. hymenoides	0.1 (0.8) a		
A. Smithii	6.8 (5.3) b		
A. Gerardii	43.0 (12.3) cd		
B. gracilis	92.1 (5.8) f		
E. canadensis	46.2 (14.1) d		
E. trichoides	77.4 (9.3) e		
P. virgatum	4.6 (4.1) ab		
S. nutans	41.1 (9.2) c		
ANOVA[1]			
Factor	MS	F-ratio	p-value
TNT-exposure	266.2	4.11	0.007
Species No	26087.0	402.75	<0.001
TNT-exposure x Species No	133.9	2.07	0.006

1 ANOVA results of germination percentage data, using target explosives concentration, species, and their interaction as factors (species entered as numbers in the analysis). Values that are followed by the same letter are not significantly different according to Fisher's least significant difference procedure. Underlining marks a statistically significant effect.

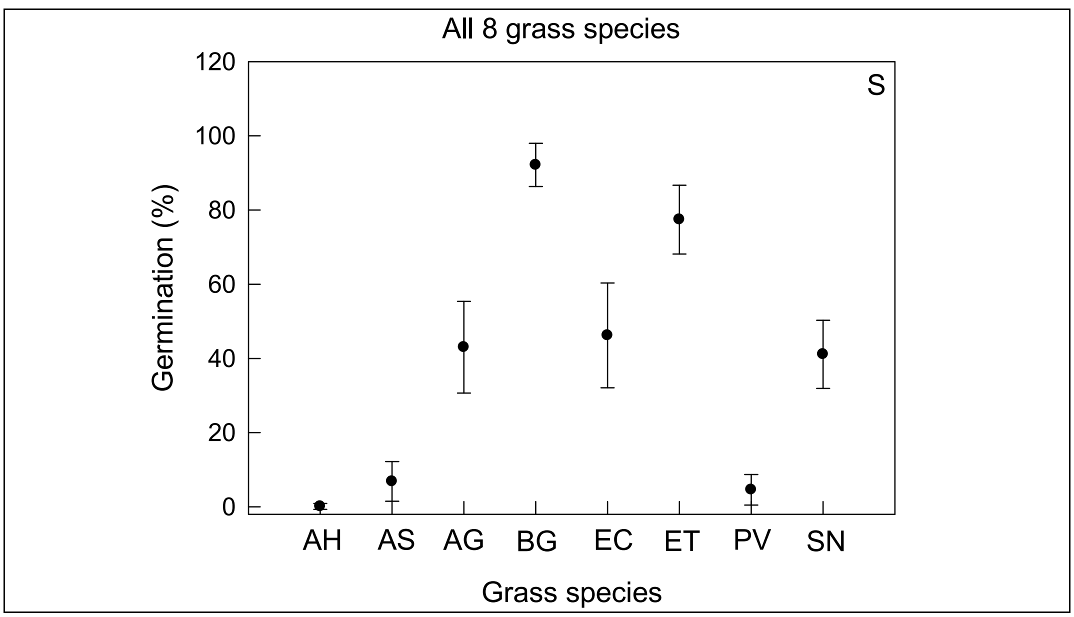

Figure 1. Germination of candidate grass species in response to 14 days of exposure to TNT-contaminated soil. Mean values and standard deviations. S is statistically significant.

TNT exposure was *E. canadensis*, in which 10-mg TNT kg^{-1} stimulated germination (Table 6, Figure 2).

RDX exposures

Germination of the grass seeds included in the RDX exposure experiment was not significantly affected by RDX concentration (p=0.861), but it was significantly affected by species (p<0.001) and by their interaction (p<0.001; Table 7). The block effects were not significant (p=0.238); therefore, all data were statistically analyzed as if completely randomized. The solvent effect was also not significant (p=0.217); therefore, both the methanol-spiked references and the water controls were included in the dataset for further analysis. Because the interaction term was significant, the overall RDX exposure effect could not be separated from the species effect. Germination confirmed the pattern found in the previous TNT exposures, in that it greatly differed with species, being very poor in *A. hymenoides*, low in *A. Smithii* and *P. virgatum*, intermediate in *A. Gerardii*, *E. canadensis*, and *S. nutans*, and significantly higher than in all other grasses in *B. gracilis* and *E. trichoides* (Table 7; Figure 3). The species in which germination was significantly affected by RDX exposure were *A. Gerardii*, *B. gracilis*, *E. trichoides,* and *P. virgatum* (Table 8, Figure 4). Germination was inhibited by $\geq$ 100-mg RDX kg^{-1} in *A. Gerardii* and $\geq$ 1,000-mg RDX kg^{-1} in *B. gracilis*. In contrast, germination was stimulated by 100-mg RDX kg^{-1} in *E. trichoides* and 500-mg kg^{-1} in *P. virgatum*.

Forbs

TNT exposures

Germination of the forb seeds included in the TNT-exposure experiment was analyzed in data pertaining to exposures lasting 4 to 12 days (4-12-d). In this experiment a completely randomized experimental design had been followed. Germination was not significantly affected by TNT concentration (p=0.324), but it was significantly affected by species (p<0.001) and by their interaction (p=0.001; Table 9). The solvent effect was not significant (p=0.627); therefore, both the methanol-spiked references and the water controls were included in the dataset for further analysis. As in the grasses, the interaction term was significant; therefore, the overall TNT exposure effect could not be separated from the species effect. Germination was far lower than in grasses, and greatly differed with species, being very poor in

Table 6. Germination of individual grass species in response to 14 days of exposure to TNT-contaminated soil. Mean values and standard deviations are shown (N=7). ANOVA results[1] are listed.

TNT exposure grasses								
Factor	Germination (%)							
TNT exposure	*A. hymenoides*	*A. Smithii*	*A. Gerardii*	*B. gracilis*	*E. canadensis*	*E. trichoides*	*P. virgatum*	*S. nutans*
Control	0.4 (1.3) a	6.4 (5.7) a	47.2 (16.7) a	94.0 (5.0) a	44.4 (12.8) a	79.6 (9.1) a	4.0 (4.2) a	45.2 (5.3) b
10-mg kg^{-1} TNT	0 (0) a	10.4 (6.1) a	44.0 (7.5) a	91.2 (5.9) a	63.2 (10.0) b	80.0 (7.5) a	6.4 (3.5) a	34.4 (12.1) a
50-mg kg^{-1} TNT	0 (0) a	4.8 (4.4) a	40.0 (4.9) a	92.8 (5.2) a	45.6 (6.7) a	72.0 (13.9) a	2.4 (3.5) a	42.4 (9.6) ab
100-mg kg^{-1} TNT	0 (0) a	6.4 (5.7) a	36.8 (10.0) a	88.8 (7.7) a	33.6 (10.8) a	76.0 (4.9) a	6.4 (4.5) a	38.4 (9.6) ab
ANOVA[1]								
Factor	MS	F-ratio	p-value					
TNT-exposure AH	0.32	0.47	0.708					
TNT-exposure AS	29.01	1.01	0.406					
TNT-exposure AG	1231.67	0.72	0.675					
TNT-exposure BG	1895.19	1.29	0.309					
TNT-exposure EC	2272.96	6.29	<u>0.003</u>					
TNT-exposure ET	79.25	0.91	0.455					
TNT-exposure PV	20.05	1.22	0.327					
TNT exposure SN	145.81	1.92	0.157					

[1] ANOVA results of germination percentage data, using target explosives concentration as factor. Values that are followed by the same letter are not significantly different according to Fisher's least significant difference procedure. Underlining marks a statistically significant effect.

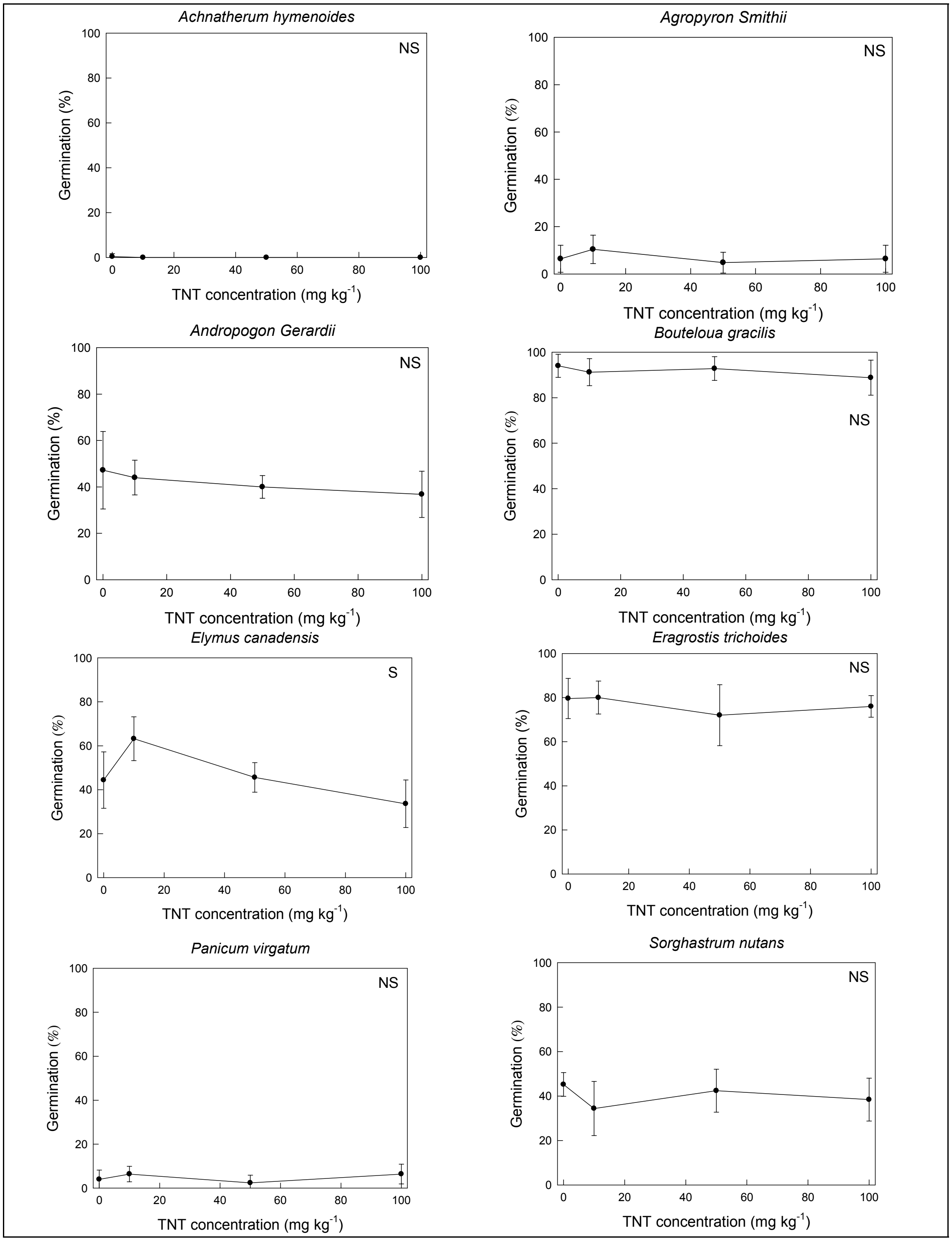

Figure 2. Germination of individual grass species in response to 14 days of exposure to TNT-contaminated soil. Mean values and standard deviations. S is statistically significant, NS is not statistically significant.

Table 7. Germination of candidate grass species in response to 13 days of exposure to RDX-contaminated soil. Mean values and standard deviations are shown (N=7). ANOVA results[1] are listed.

RDX Exposure Grasses			
Species	Germination (%)		
A. hymenoides	0.1 (0.8) a		
A. Smithii	7.4 (6.6) b		
A. Gerardii	42.2 (10.2) d		
B. gracilis	90.2 (10.8) f		
E. canadensis	37.1 (11.8) c		
E. trichoides	76.6 (11.2) e		
P. virgatum	5.4 (5.5) b		
S. nutans	42.4 (9.1) d		
ANOVA[1]			
Factor	MS	F-ratio	p-value
RDX-exposure	32.7	0.53	0.661
Species No	24664.9	400.34	<u><0.001</u>
RDX-exposure x Species No	243.6	3.95	<u><0.001</u>

[1] ANOVA results of germination percentage data, using target explosives concentration, species, and their interaction as factors (species entered as numbers in the analysis). Values that are followed by the same letter are not significantly different according to Fisher's least significant difference procedure. Underlining marks a statistically significant effect.

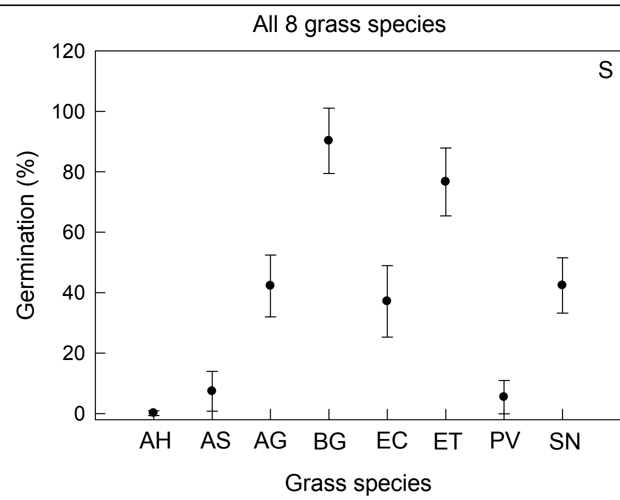

Figure 3. Germination of candidate grass species in response to 13 days of exposure to RDX-contaminated soil. Mean values and standard deviations. S is statistically significant.

Table 8. Germination of individual grass species in response to 13 days of exposure to RDX-contaminated soil. Mean values and standard deviations are shown (N=7). ANOVA results[1] are listed.

RDX Exposure Grasses								
Factor	Germination (%)							
RDX Exposure	*A. hymenoides*	*A. Smithii*	*A. Gerardii*	*B. gracilis*	*E. canadensis*	*E. trichoides*	*P. virgatum*	*S. nutans*
Control	0 (0) a	6.0 (3.8) a	50.8 (7.0) b	95.2 (4.5) b	34.8 (13.7) a	73.2 (9.8) a	3.2 (2.5) ab	42.8 8.8) a
100-mg kg^{-1} RDX	0 (0) a	6.4 (8.2) a	40.0 (7.5) a	92.0 (2.8) b	33.6 (16.6) a	89.6 (6.6) b	0.8 (1.7) a	36.0 (6.9) a
500-mg kg^{-1} RDX	0 (0) a	9.6 (7.2) a	32.0 (5.6) a	94.4 (4.5) b	38.4 (6.0) a	74.4 (12.8) a	12.8 (5.2) c	44.0 (9.3) a
1000-mg kg^{-1} RDX	0.8 (1.7) a	8.8 (9.5) a	37.6 (9.2) a	74.4 (15.1) a	44.0 (4.0) a	72.8 (8.1) a	7.2 (5.2) b	46.4 (10.8) a
ANOVA[1]								
Factor	MS	F-ratio	p-value					
RDX-exposure AH	0.85	1.40	0.270					
RDX-exposure AS	19.52	0.42	0.743					
RDX-exposure AG	463.25	8.53	<0.001					
RDX-exposure BG	534.18	9.23	<0.001					
RDX-exposure EC	120.21	0.84	0.488					
RDX-exposure ET	352.32	3.75	0.026					
RDX-exposure PV	148.05	10.80	<0.001					
RDX exposure SN	99.73	1.22	0.327					

[1] ANOVA results of germination percentage data, using target explosives concentration as factor. Values that are followed by the same letter are not significantly different according to Fisher's least significant difference procedure. Underlining marks a statistically significant effect.

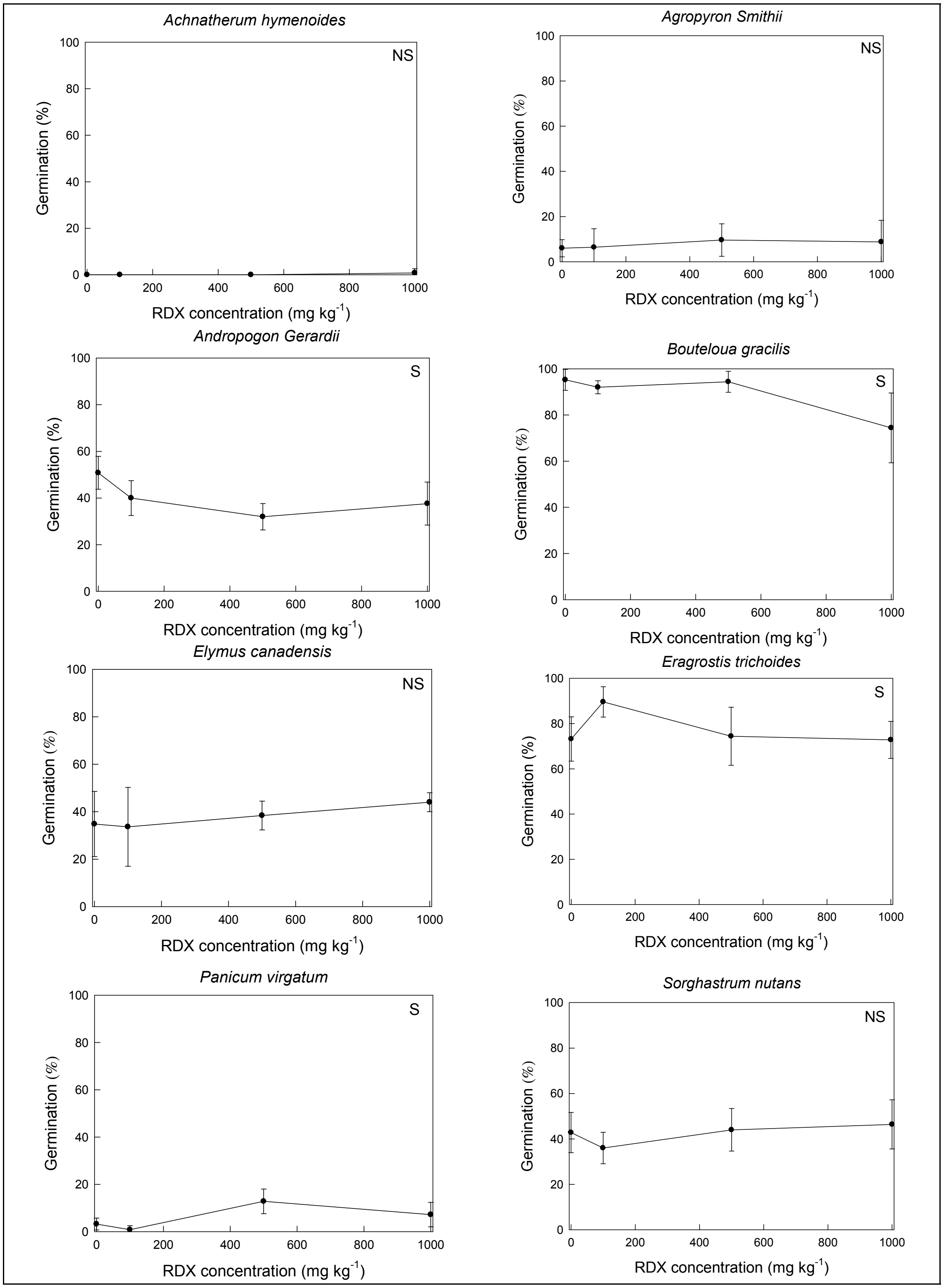

Figure 4. Germination of individual grass species in response to 13 days of exposure to RDX-contaminated soil. Mean values and standard deviations. S is statistically significant, NS is not statistically significant.

Table 9. Germination of candidate forb species in response to 4-12 days of exposure to TNT-contaminated soil. Mean values and standard deviations are shown (N=7). ANOVA results[1] are listed.

TNT Exposure Forbs			
Species	Germination (%)		
A.millefolium	0.5 (1.3) a		
A. retroflexus	4.3 (4.8) a		
A. syriaca	22.8 (8.3) b		
D. stramonium	0 (0) a		
I. lacunosa	30.2 (19.1) c		
P. pensylvanicum	0 (0) a		
P. oleracea	23.6 (7.3) b		
S. spinosa	2.7 (4.9) a		
ANOVA[1]			
Factor	MS	F-ratio	p-value
TNT-exposure	68.3	1.17	0.324
Species No	3513.4	59.90	<u><0.001</u>
TNT-exposure x Species No	141.8	2.42	<u>0.001</u>

[1] ANOVA results of germination percentage data, using target explosives concentration, species, and their interaction as factors (species entered as numbers in the analysis). Values that are followed by the same letter are not significantly different according to Fisher's least significant difference procedure. Underlining marks a statistically significant effect.

A. millefolium, D. stramonium, and *P. pensylvanicum*, low in *A. retroflexus* and *S. spinosa*, and intermediate in *A. syriaca*, *I. lacunosa*, and *P. oleracea* (Table 9, Figure 5). In none of the species germination was affected significantly by TNT exposure (Table 10, Figure 6).

RDX exposures

Germination of the forb seeds included in the RDX exposure experiment was also analyzed in data pertaining to 4-12-d exposures. This experiment also followed a completely randomized experimental design. Germination was not significantly affected by RDX concentration (p=0.125), but it was significantly affected by species (p<0.001; Table 11). The solvent effect was also significant (p=0.025); therefore, only the methanol-spiked references were included in the dataset for further analysis, omitting the water controls. Germination confirmed the pattern found in the previous TNT

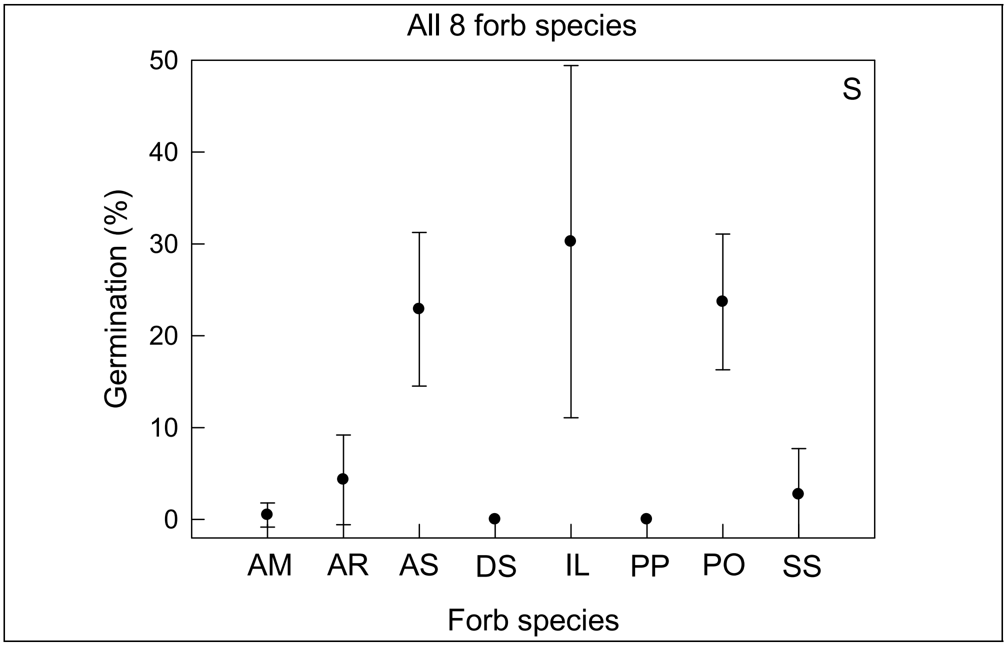

Figure 5. Germination of candidate grass species in response to 4-12 days of exposure to TNT-contaminated soil. Mean values and standard deviations. S is statistically significant.

exposures, in that it greatly differed with species, being very poor in *A. millefolium, D. stramonium,* and *P. pensylvanicum*, low in *A. retroflexus* and *S. spinosa*, and intermediate in *A. syriaca*, *I. lacunosa*, and *P. oleracea* (Table 11; Figure 7). Germination was significantly stimulated by concentrations of $\geq$ 1,000-mg RDX kg^{-1} in *P. oleracea* and *S. spinosa* (Table 12, Figure 8).

Conclusions and recommendations for research

1. Of the eight grasses screened, five exhibited medium to good germination capacity.
2. Of the two good-germinating grasses, neither was significantly inhibited by TNT up to a concentration of 100 mg kg^{-1} soil; and one species was stimulated by a low TNT concentration of 10 mg kg^{-1} soil (*Elymus canadensis*).
3. Of the two good-germinating grasses, one was significantly inhibited by a high RDX concentration of $\geq$1,000 mg kg^{-1} soil (*Bouteloua gracilis*).
4. Of the eight forbs screened, five exhibited low to medium germination capacity.

Table 10. Germination of individual forb species in response to 4-12 days of exposure to TNT-contaminated soil. Mean values and standard deviations are shown (N=7). ANOVA results[1] are listed.

TNT Exposure Forbs								
Factor	Germination (%)							
TNT Exposure	*A. millefolium*	*A. retroflexus*	*A. syriaca*	*D. stramonium*	*I. lacunosa*	*P. pensylvanicum*	*P. oleracea*	*S. spinosa*
Control	0 (0)	6.4 (5.0) b	18.8 (5.6) a	0	41.2 (20.8) b	0	22.4 (8.6) a	2.4 (4.2) a
10-mg kg^{-1} TNT	0 (0)	4.0 (3.2) ab	26.0 (7.6) ab	0	17.0 (12.8)	0	24.0 (5.6) a	4.0 (4.6) a
50-mg kg^{-1} TNT	1.6 (2.1)	5.0 (7.5) ab	25.0 (5.0) ab	0	24.0 (17.2) ab	0	22.0 (6.9) a	1.0 (2.0) a
100-mg kg^{-1} TNT	0.8 (1.7)	1.0 (2.0) a	27.0 (14.3) b	0	32.0 (16.3) ab	0	30.0 (6.9) a	2.0 (2.3) a
ANOVA[1]								
Factor	MS	F-ratio	p-value					
TNT-exposure AM	NA[2]							
TNT-exposure AR	37.54	1.71	0.195					
TNT-exposure AS	120.21	1.91	0.158					
TNT-exposure DS	NA[2]							
TNT-exposure IL	864.3	2.91	0.058					
TNT-exposure PP	NA[2]							
TNT-exposure PO	26.45	0.45	0.723					
TNT exposure SS	9.81	0.37	0.778					

[1] ANOVA results of germination percentage data, using target explosives concentration as factor. Values that are followed by the same letter are not significantly different according to Fisher's least significant difference procedure. Underlining marks a statistically significant effect. [2] NA, not applicable: *D. stramonium* and *P. pensylvanicum* did not germinate; insufficient data for analysis of *A. millefolium*

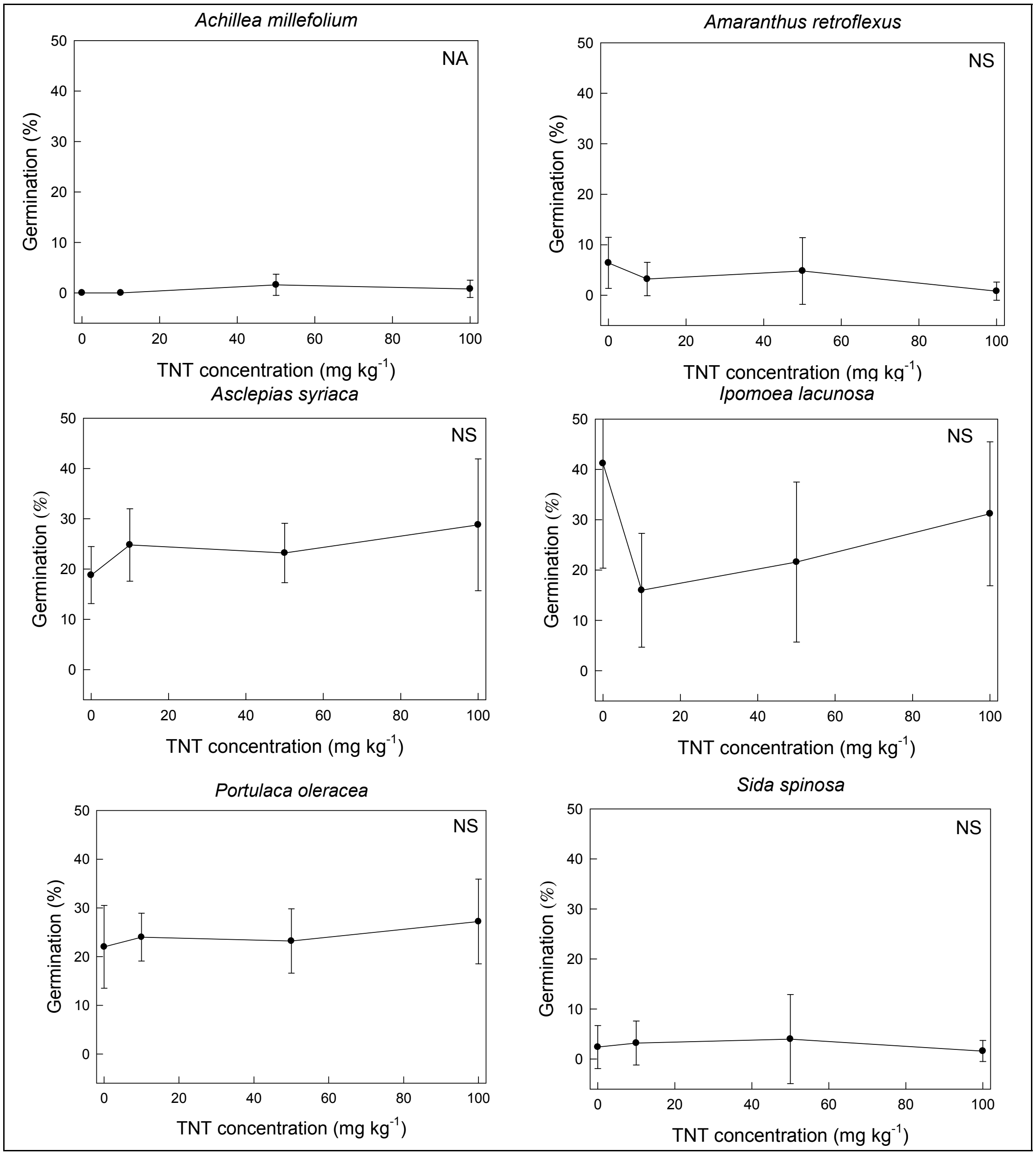

Figure 6. Germination of individual forb species in response to 4-12 days of exposure to TNT-contaminated soil. *D. stramonium* and *P. pensylvanicum* did not germinate. Mean values and standard deviations. S is statistically significant, NS is not statistically significant, NA is not applicable (insufficient data).

Table 11. Germination of candidate forb species in response to 4-12 days of exposure to RDX-contaminated soil. Mean values and standard deviations are shown (N=7). Water controls excluded. ANOVA results[1] are listed.

RDX Exposure Forbs			
Species	Germination (%)		
A.millefolium	0 (0) a		
A. retroflexus	2.2 (4.5) a		
A. syriaca	60.0 (12.6) b		
D. stramonium	0 (0) a		
I. lacunosa	30.0 (16.2) d		
P. pensylvanicum	0 (0) a		
P. oleracea	22.6 (17.5) c		
S. spinosa	6.4 (7.5) b		
ANOVA[1]			
Factor	MS	F-ratio	p-value
RDX-exposure	169.2	1.95	0.125
Species No	9201.0	105.88	<u><0.001</u>
RDX-exposure x Species No	178.5	2.05	<u>0.007</u>

[1] ANOVA results of germination percentage data, using target explosives concentration, species, and solvent type as factors (species entered as numbers in the analysis). Values that are followed by the same letter are not significantly different according to Fisher's least significant difference procedure. Underlining marks a statistically significant effect.

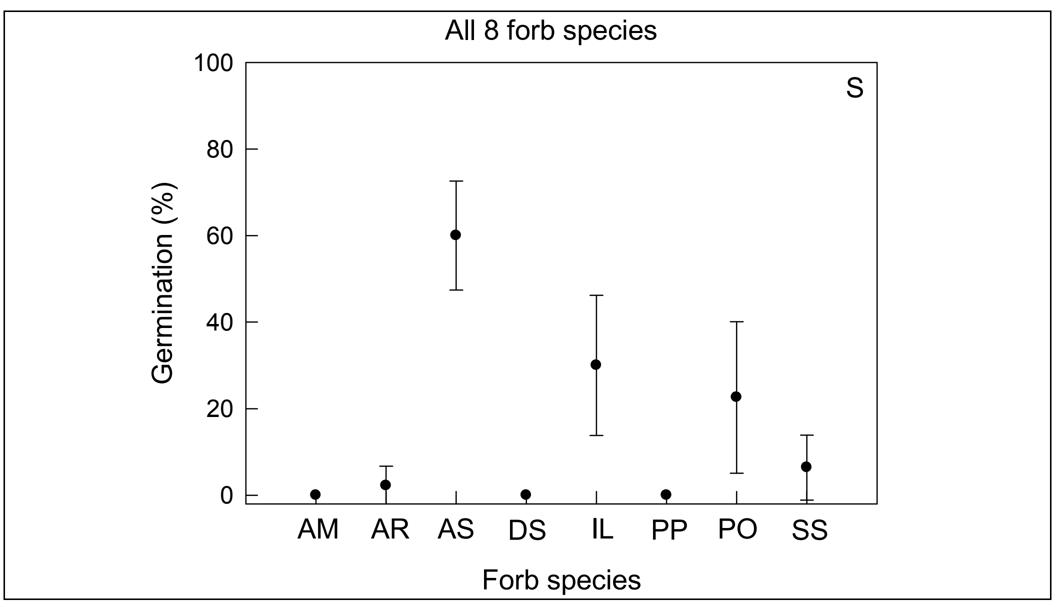

Figure 7. Germination of candidate forb species in response to 4-12 days of exposure to RDX-contaminated soil. Mean values and standard deviations. S is statistically significant.

Table 12. Germination of individual forb species in response to 4-12 days of exposure to RDX-contaminated soil. Mean values and standard deviations are shown (N=7). Water controls excluded.

RDX exposure forbs					
Factor	Germination (%)				
RDX exposure	*A. retroflexus*	*A. syriaca*	*I. lacunosa*	*P. oleracea*	*S. spinosa*
Control	0 (0) a	8.0 (7.4) a	39.2 (5.2) a	6.4 (5.3) a	2.4 (2.1) ab
100-mg kg^{-1} RDX	0.8 (1.7) a	12.8 (12.4) a	26.4 (19.7) a	24.8 (17.2) ab	0 (0) a
500-mg kg^{-1} RDX	3.2 (5.2) a	0 (0) a	26.4 (24.4) a	20.0 (18.1) a	8.0 (6.3) b
1000-mg kg^{-1} RDX	4.8 (7.1) a	12.0 (20.8) a	28.8 (9.5) a	39.2 (10.7) b	15.2 (7.1) c
ANOVA[1]					
Factor	MS	F-ratio	p-value		
RDX-exposure AR	24.26	1.19	0.345		
RDX-exposure AS	171.46	1.07	0.391		
RDX-exposure IL	186.40	0.68	0.579		
RDX-exposure PO	916.00	4.75	<u>0.014</u>		
RDX exposure SS	228.26	9.51	<u><0.001</u>		

[1] ANOVA results of germination percentage data, using target explosives concentration as factor. Water controls excluded. Values that are followed by the same letter are not significantly different according to Fisher's least significant difference procedure. Underlining marks a statistically significant effect.

5. Forbs germinated less synchronously and often earlier than grasses.
6. Of the three medium-germinating forbs, none was significantly inhibited by TNT up to a concentration of 100 mg kg^{-1} soil.
7. Of the three medium-germinating forbs, none was significantly inhibited by RDX; one species was stimulated by a high RDX concentration of $\geq$1,000 mg kg^{-1} soil (*Portulaca oleracea*).

Based on the results of the short-term screening experiment, five grasses and five forbs were identified as rapidly colonizing and short-term tolerant towards TNT- and RDX-contamination of soils (Table 13). These species are: the grasses, *Andropogon Gerardii*, *Bouteloua gracilis*, *Elymus canadensis*, *Eragrostis trichoides*, *Sorghastrum nutans*; and the forbs: *Amaranthus retroflexus*, *Asclepias syriaca*, *Ipomoea lacunosa*, *Portulaca oleracea*, *Sida spinosa*.

This study provides data that can be used as a basis for the identification of herbaceous plants with the capacity to rapidly colonize TNT- and RDX-contaminated soils. All these species are listed in the literature as being resilient and are, therefore, considered as potentially suitable to persist on ranges. Subsequent research will further elucidate whether these species (1) may persist on the long(er) term when in contact with energetics-contaminated soil; (2) exclude, absorb, accumulate, and/or metabolize energetics; (3) produce potentially toxic metabolites; and (4) may inhibit energetics leaching from soils by treatment/containment. It is expected that the results of these studies will support the selection of herbaceous plant species that can be successfully used for phytoremediation (containment and/or phytoextraction).

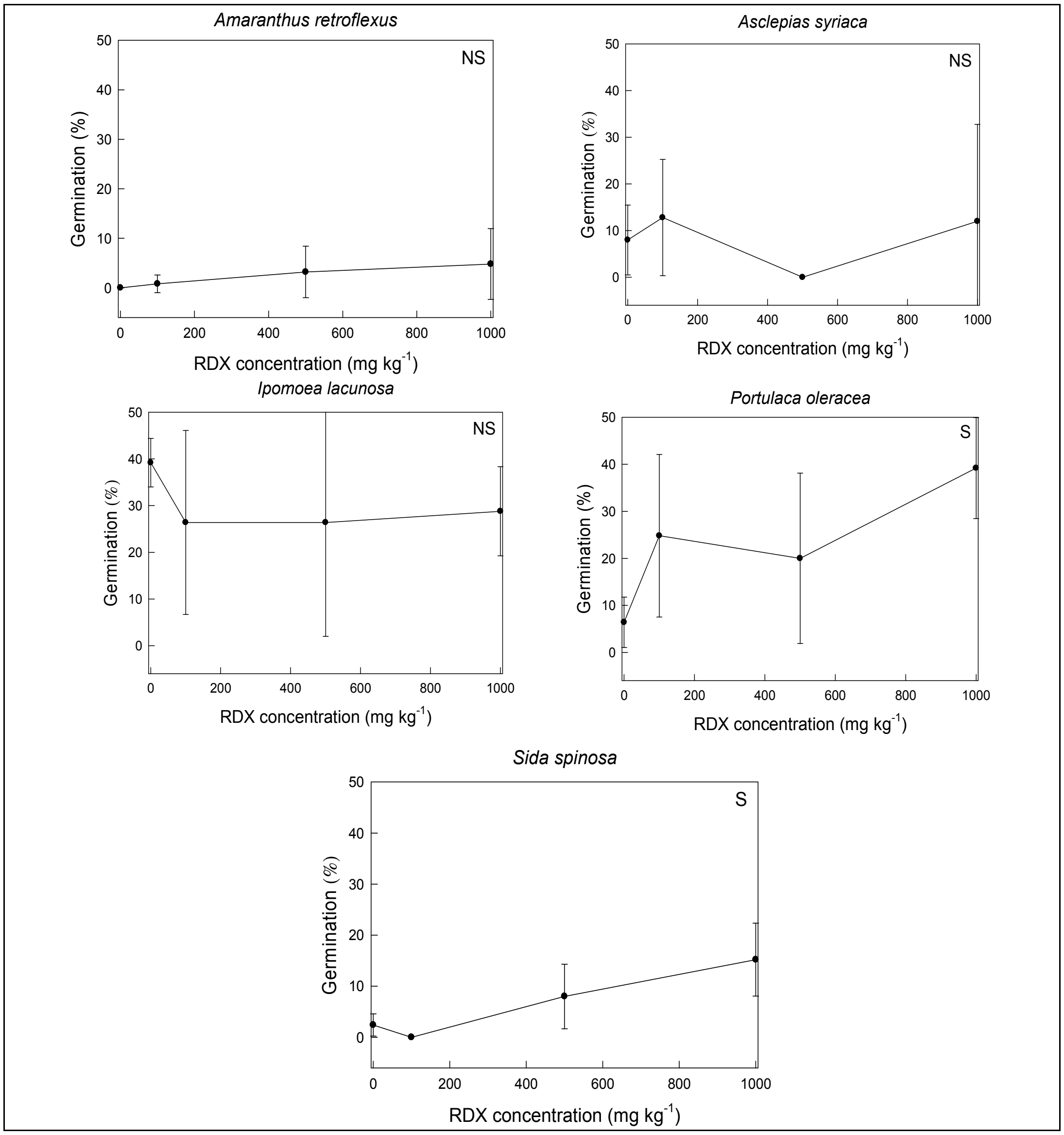

Figure 8. Germination of individual forb species in response to 4-12 days of exposure to RDX-contaminated soil. *A. millefolium*, *D. stramonium*, and *P. pensylvanicum* did not germinate. Mean values and standard deviations. S is statistically significant, NS is not statistically significant.

Table 13. Germination capacity and tolerance towards TNT and RDX based on the results of the short-term screening experiments. Species with a considerable germination capacity and identified as short-term tolerant are marked.*

Plant Species	Germination Capacity	TNT Tolerance	RDX Tolerance
Grass			
Achnatherum hymenoides	Very poor	NS	NS
Agropyron Smithii	Low	NS	NS
*Andropogon Gerardii**	Medium	NS	Inhibition by $\geq$100 mg kg^{-1}
*Bouteloua gracilis**	Good	NS	Inhibition by $\geq$1,000 mg kg^{-1}
*Elymus canadensis**	Medium	Stimulation by 10 mg kg^{-1}	NS
*Eragrostis trichoides**	Good	NS	Stimulation by 100 mg kg^{-1}
Panicum virgatum	Low	NS	Stimulation by 500 mg kg^{-1}
*Sorghastrum nutans**	Medium	NS	NS
Forb			
Achillea millefolium	Very poor	-	-
*Amaranthus retroflexus**	Low	NS	NS
*Asclepias syriaca**	Medium	NS	NS
Datura stramonium	Very poor	-	-
*Ipomoea lacunosa**	Medium	NS	NS
Polygonum pensylvanicum	Very poor	-	-
*Portulaca oleracea**	Medium	NS	Stimulation by $\geq$1,000 mg kg^{-1}
*Sida spinosa**	Low	NS	Stimulation by $\geq$1,000 mg kg^{-1}

NS is not statistically significant.

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14. ABSTRACT
This report identifies rapidly colonizing and resilient grasses/forbs that are tolerant to range-relevant contaminants, with emphasis on TNT and RDX. A literature review identified herbaceous plant species with characteristics that make them potential candidates for use on ranges for phytostabilization and phytoextraction purposes. The 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. Plant species reviewed included 64 grasses and 61 forbs. Based on initial review, eight grasses and eight forbs were selected for tolerance testing. Short-term screening experiments were conducted to evaluate the phytotoxicity of TNT- and RDX-spiked artificial soils to the plants. Seeds were exposed in the laboratory and germination was used as a parameter for plant response. Based on results of this experiment, five grasses and five forbs were identified as rapidly colonizing and short-term tolerant towards TNT- and RDX-contamination of soils.

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