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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 $\leq 0.05$).

## 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 | <u>0.007</u> |
| Species No | 26087.0 | 402.75 | <u><0.001</u> |
| TNT-exposure x Species No | 133.9 | 2.07 | <u>0.006</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.

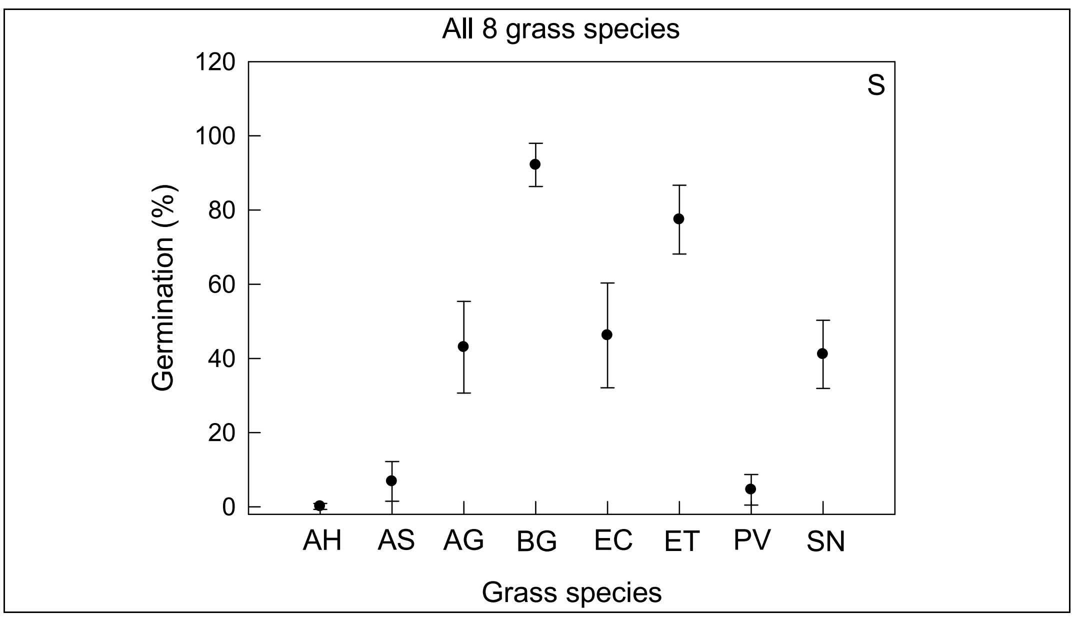


Figure 1. Germination of candidate grass species in response to 14 days of exposure to TNT-contaminated soil. Mean values and standard deviations. S is statistically significant.

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.

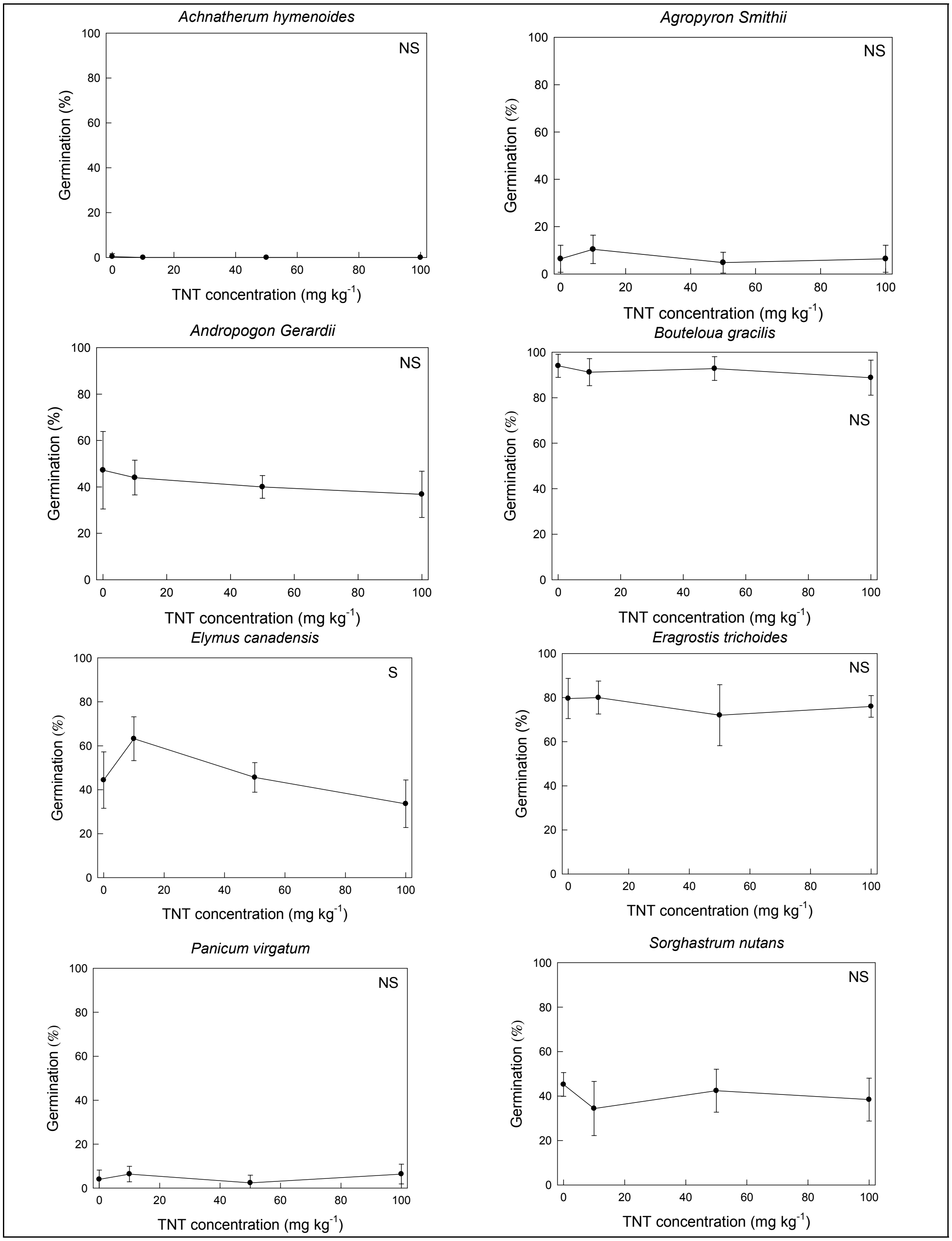


Figure 2. Germination of individual grass species in response to 14 days of exposure to TNT-contaminated soil. Mean values and standard deviations. S is statistically significant, NS is not statistically significant.

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.

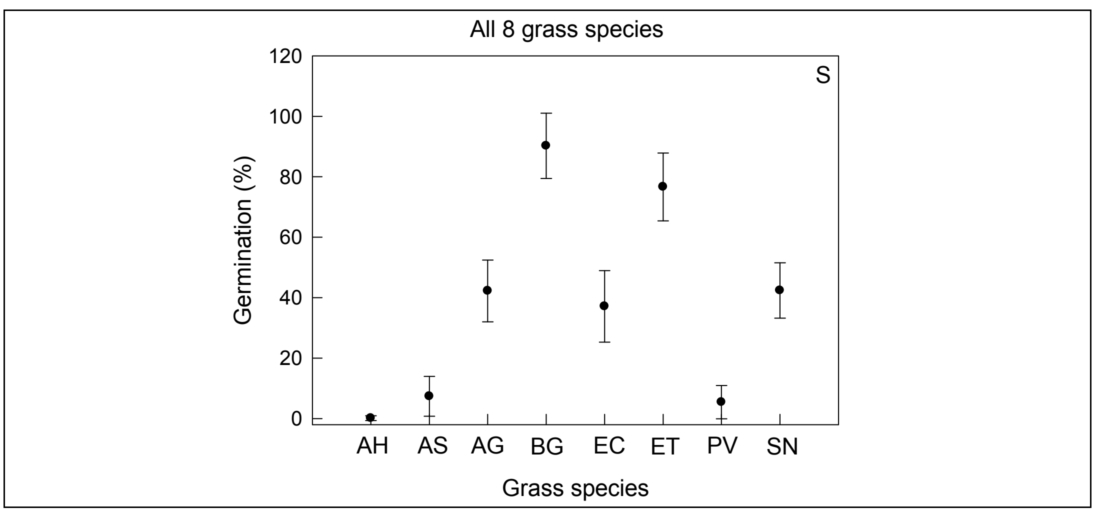


Figure 3. Germination of candidate grass species in response to 13 days of exposure to RDX-contaminated soil. Mean values and standard deviations. S is statistically significant.

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.

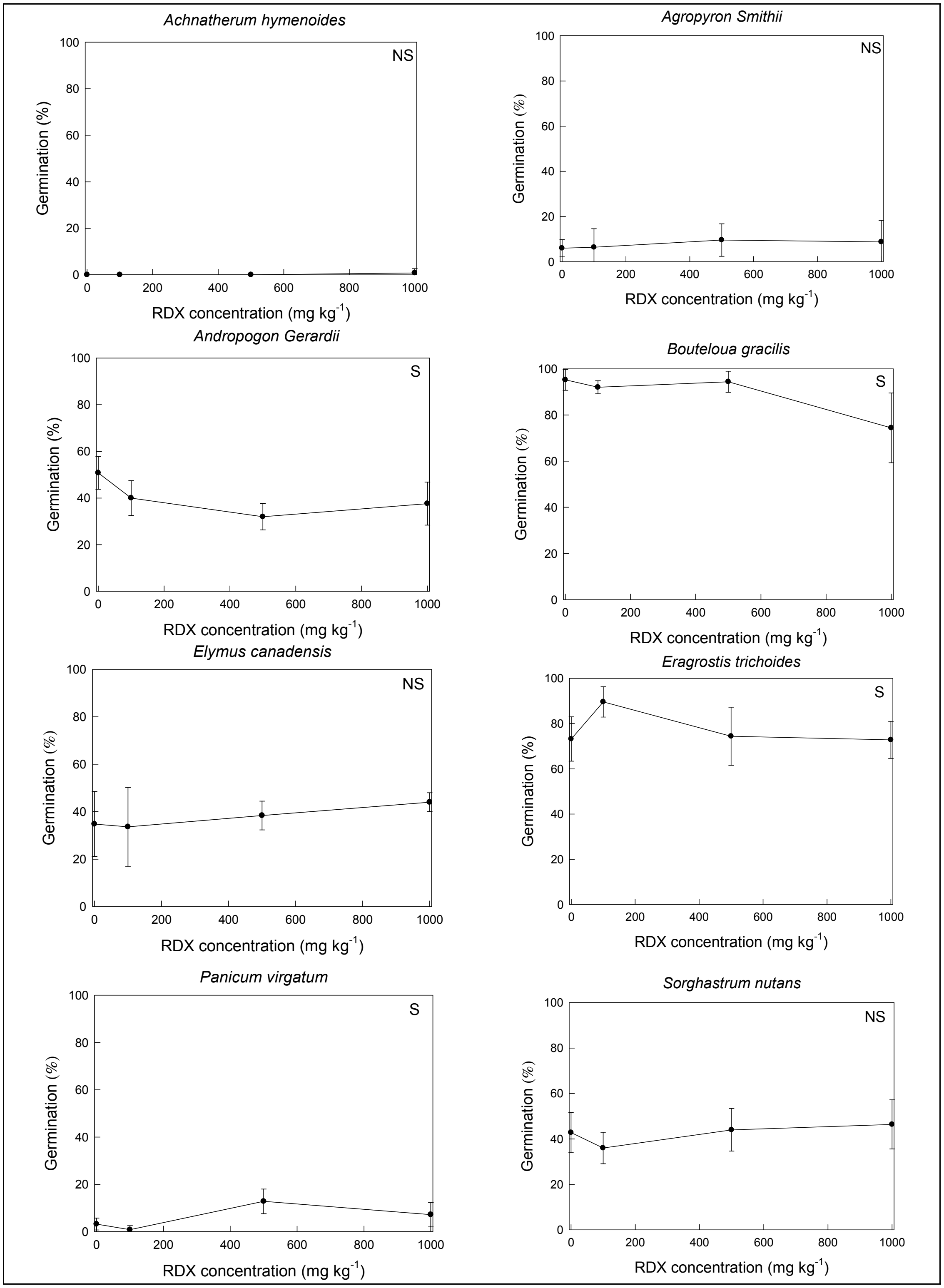


Figure 4. Germination of individual grass species in response to 13 days of exposure to RDX-contaminated soil. Mean values and standard deviations. S is statistically significant, NS is not statistically significant.

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

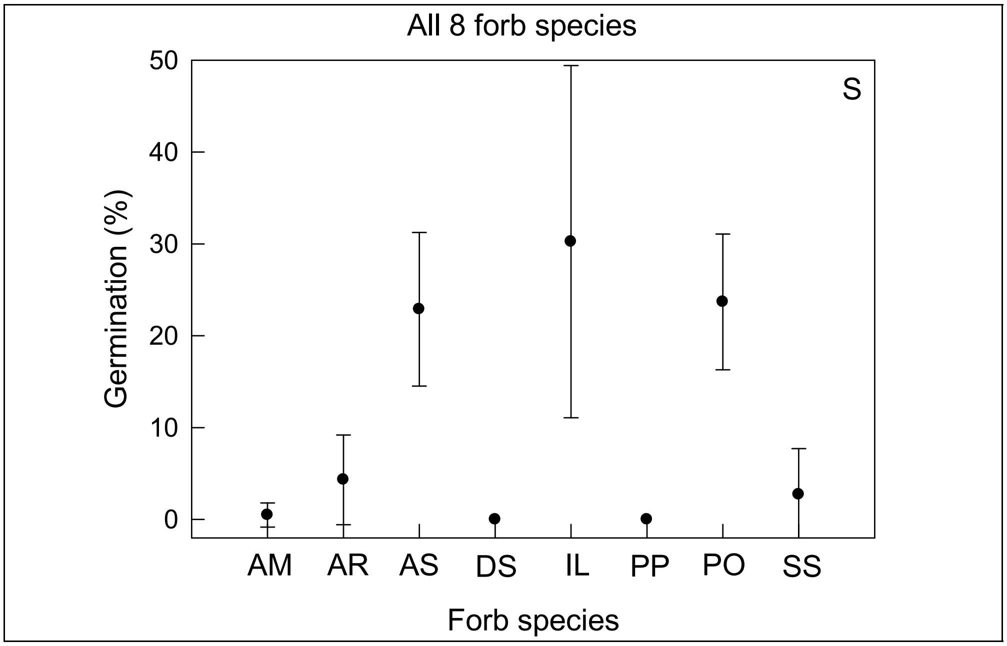


Figure 5. Germination of candidate grass species in response to 4-12 days of exposure to TNT-contaminated soil. Mean values and standard deviations. S is statistically significant.

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*

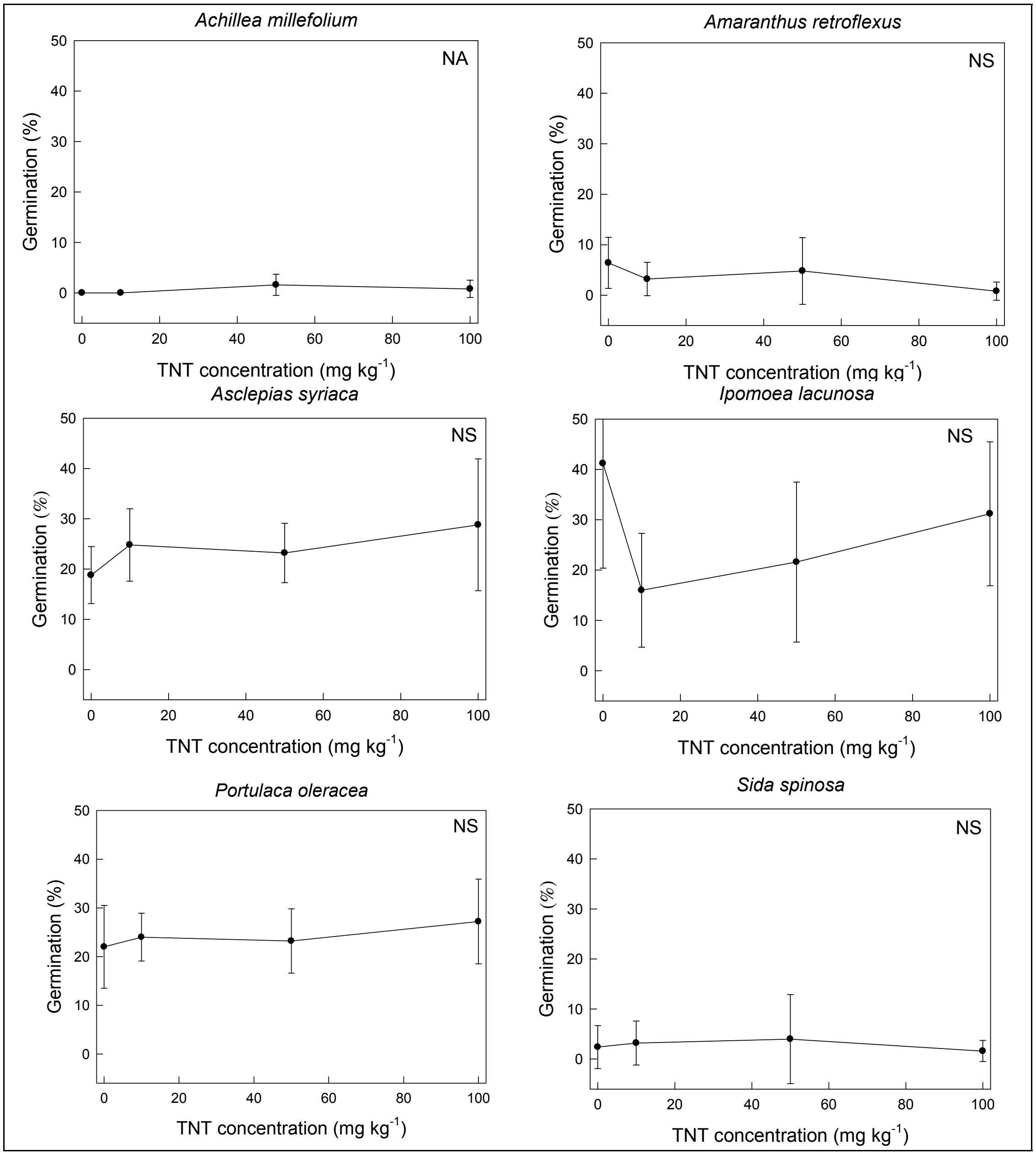


Figure 6. Germination of individual forb species in response to 4-12 days of exposure to TNT-contaminated soil. *D. stramonium* and *P. pensylvanicum* did not germinate. Mean values and standard deviations. S is statistically significant, NS is not statistically significant, NA is not applicable (insufficient 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 | <0.001 |
| RDX-exposure x Species No | 178.5 | 2.05 | 0.007 |

[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.

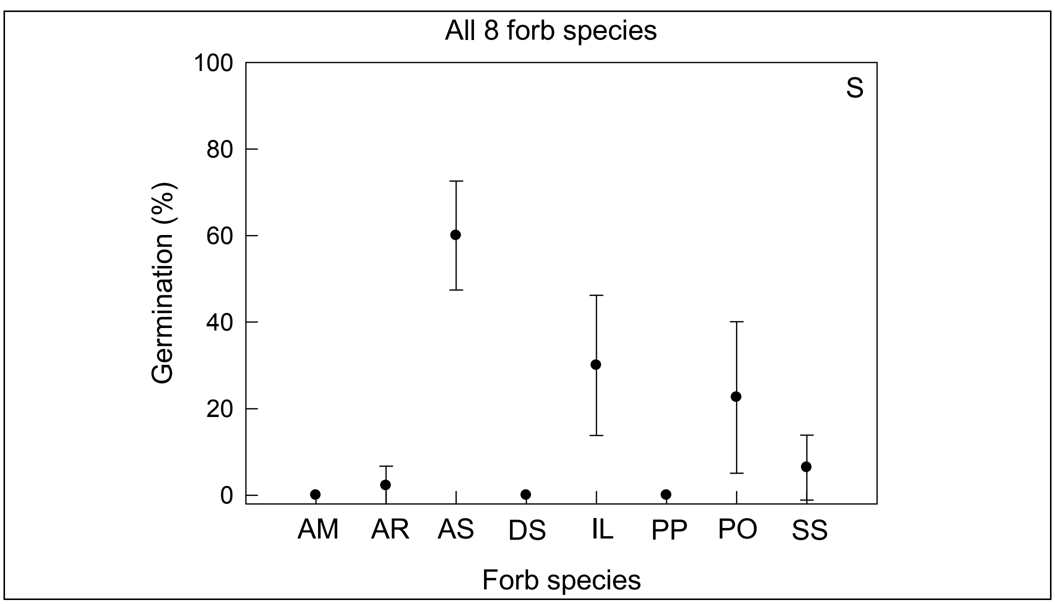


Figure 7. Germination of candidate forb species in response to 4-12 days of exposure to RDX-contaminated soil. Mean values and standard deviations. S is statistically significant.

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).

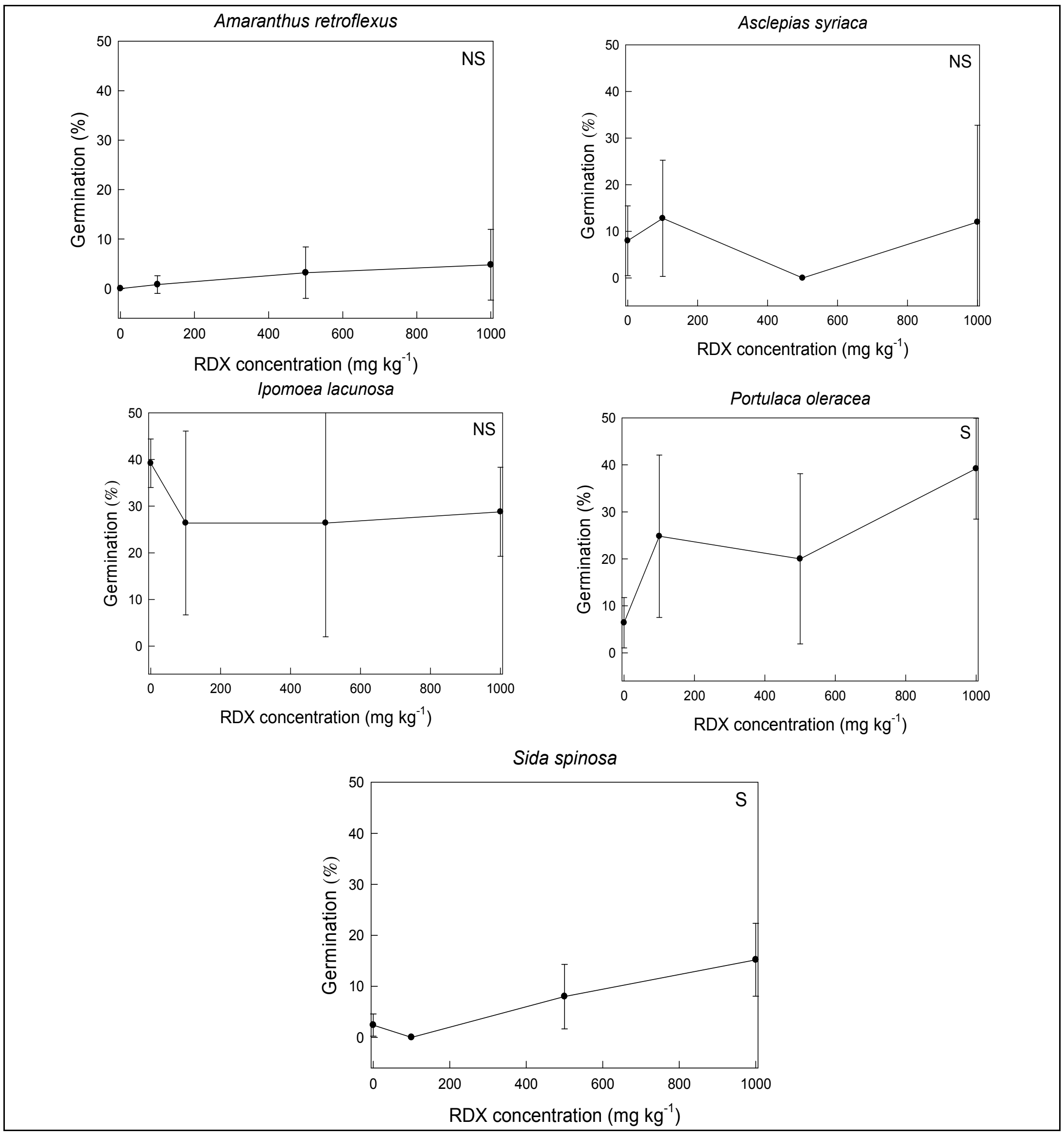


Figure 8. Germination of individual forb species in response to 4-12 days of exposure to RDX-contaminated soil. *A. millefolium*, *D. stramonium*, and *P. pensylvanicum* did not germinate. Mean values and standard deviations. S is statistically significant, NS is not statistically significant.

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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