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Phytophotolysis of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in Leaves of Reed Canary Grass

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Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) was degraded in reed canary grass leaves exposed to simulated sunlight to primary products nitrous oxide and 4-nitro-2,4-diazabutanal. This is the first time that 4-nitro-2,4-diazabutanal, a potentially toxic degradate, has been measured in plant tissues following phytotransformation of RDX. These compounds, along with nitrite and formaldehyde, were also detected in aqueous RDX systems exposed to the same simulated sunlight. Results showed that the initial products of RDX photodegradation in translucent plant tissues were similar to products formed from aqueous photolysis of RDX. Combustion analysis of leaves following ^{14}C-RDX uptake and subsequent light exposure revealed the presence of tissue-bound material that could not be extracted with acetonitrile. No detectable formaldehyde was emitted from the leaves. The detection of similar RDX degradation products in both aqueous and plant-based systems suggests that RDX may be initially transformed by similar mechanisms in both systems. Direct photolysis of RDX via ultraviolet irradiation passing into the leaves is hypothesized to be responsible for the observed transformations. In addition, membrane-bound "trap chlorophyll" in the chloroplasts may shuttle electrons to RDX as an indirect photolysis transformation mechanism. Results from this study indicate that reed canary grass facilitates photochemical degradation of RDX, and this mechanism should be considered along with more established phytoremediation processes when assessing the fate of contaminants in plant tissues. Plant-mediated phototransformation of xenobiotic compounds is a process that may be termed "phytophotolysis".

Introduction

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) continues to be a major component in a majority of munitions in use by military forces worldwide. The resulting water and soil contamination at testing sites, manufacturing plants, and storage facilities has created potential hazards for human and ecological health. Efforts to address these potential hazards have involved a variety of treatment strategies including bioremediation (*1*−*12*), zerovalent iron (*13*), chemical degradation (*14*−*17*), thermal decomposition (*18*, *19*), photodegradation (*20*−*24*), and phytoremediation (*25*−*32*).

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RDX is mobile in the environment, and it is taken up and translocated to shoots and leaves very quickly (*29*). RDX uptake studies with plants indicate the formation of non-volatile residues and release of volatile degradates from hybrid poplar trees (*Populus deltoides* x *nigra*, DN34), reed canary grass (*Phalaris arundinacea*), and parrot feather (*Myriophyllum aquatica*) (*28*−*30*). The "green liver" model for xenobiotic compound detoxification in plant tissues indicates that transformation, conjugation, and compartmentation (*33*) categorize the fate of a chemical such as RDX once inside the plant. The model implies transformation and conjugation to be mainly enzyme-catalyzed processes. The work presented here suggests that initial chemical transformation by plant-facilitated photolysis may play a role equal to or greater than that of enzyme-based phenomena in phytoremediation.

The results in this paper and other studies (*20*, *34*−*36*) indicate that RDX will undergo direct or indirect photolysis in aqueous solution. Most plant species are comprised of over 70% water; therefore photolytic transformations similar to those in water could occur in plant tissues, especially in leaves designed for harvesting natural sunlight. As RDX (or other photosensitive chemicals) enters the leaves via the xylem, photolysis can occur as unabsorbed ultraviolet light penetrates the tissues and transforms the xenobiotic compound. In addition, dissolved RDX may diffuse out of the xylem to plant cells and organelles where indirect photolysis processes can occur. The plant pigments could also transfer excess energy directly to RDX to facilitate photolysis. This plant-mediated, photoinduced transformation of a xenobiotic compound is a process that can be termed "phytophotolysis". Phytophotolysis contributes greatly to the degradation of RDX in reed canary grass which has been used in engineered wetlands systems for treatment of explosives-contaminated soils and surface waters. Phytophotolysis may also play a significant role in the degradation of RDX in other plant species used in phytoremediation applications throughout the world and in contaminated areas where native plants are growing in nonengineered systems.

Experimental Section

Chemicals. RDX was synthesized (*37*) in house using formaldehyde (Sigma), ammonium hydroxide (Fisher), and fuming nitric acid (Fisher). ^{13}C-RDX required ^{13}C-formaldehyde (Sigma), and ^{15}N-ring-RDX utilized ^{15}N-ammonium hydroxide (Sigma) as a synthesis precursor. All synthesized products were 99% pure or better as indicated by high-performance liquid chromatography (HPLC) with detection at 240 nm. Uniformly labeled ^{14}C-RDX was purchased from Perkin-Elmer Life and Analytical Sciences (Boston, MA) and was specified as 99% pure. The HPLC solvents were Fisher Optima grade, and Nanopure water (Barnstead, Diamond System) with greater than 18.0 MΩ-cm resistivity was used.

Reed Canary Grass. *Phalaris arundinacea* was grown from seed (Wildlife Nurseries, Inc., Oshkosh, WI) in 10% Hoagland's solution (*38*), and the plants were 6 weeks old and approximately 20 centimeters tall. The initial aqueous transpiration rate was 20.0 ± 2.3 mL/day, and the average beginning plant mass was 7.4 ± 2.6 g.

Gas Chromatography (GC). A Hewlett-Packard 5890 gas chromatograph with an electron capture detector (ECD) was used. N_2O was eluted with a GS-Q, 0.32 mm i.d., 30 m capillary column (J&W Scientific) using nitrogen carrier gas at 2 mL/min. The oven and detector temperatures were 35 and 250 °C, respectively. The data were processed using Chemstation software.

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Gas Chromatography/Mass Spectrometry (GC/MS). A Finnigan (San Jose, CA) GCQ gas chromatograph−mass spectrometer with a CTC A200S autosampler was used. $^{15}N^{14}NO$ was eluted with a GS-Q, 0.25 mm i.d., 30 m capillary column using helium carrier gas at 40 cm/s. The injection, oven, transfer line, and ion source temperatures were 200, 35, 150, and 200 °C, respectively. Full scan mode (43−48 *m*/*z*) was used.

Ion Chromatography (IC). An ion chromatography system with a Gilson 706 pump, an Alcott 728 autosampler (50 μL), a Dionex conductivity detector, and Hewlett-Packard Chemstation software was used. Nitrite was eluted with an AS4 analytical column (Dionex) with an AG4 guard column using 1.8 mM/2.0 mM carbonate/bicarbonate eluent at 1.5 mL/min.

Liquid Chromatography (LC). An Agilent (Palo Alto, CA) model 1100 high-performance liquid chromatograph with quaternary pump, vacuum degasser, autosampler, and photodiode array detector was used. RDX and 4-nitro-2,4-diazabutanal were separated with a C18 column (250 mm × 4.6 mm; Supelco part 58298, Bellefonte, PA) and methanol/water (60/40) mobile phase at 1 mL/min. Absorbance (240 nm) data were collected via Agilent Chemstation software. The formaldehyde−2,4-dinitrophenylhydrazine (DNPH) derivative was eluted with the C18 column and acetonitrile/water (55/45) at 1 mL/min. Sample injections were 100 μL.

Liquid Chromatography/Mass Spectrometry (LC/MS). A QuattroLC (Micromass Corp.) with a 2690 HPLC and a model 996 (Waters) photodiode array detector was used. Negative-ion electrospray (ES) mode with desolvation and source temperatures of 150 °C were used. Nebulizer and desolvation flows were 75 and 700 L/h, respectively. The capillary, cone, extraction, and multiplier potentials were −3 kV, −25 V, −3 V, and 650 V, respectively, and the mass resolution was 15. RDX and 4-nitro-2,4-diazabutanal were separated using a C18 column (Supelco, 15 cm × 2.1 mm, 5 micron) and acetonitrile/2 mM ammonium acetate (50/50) at 200 μL/min using 10 μL injections.

Photon Flux Sensor. A model LI-189 photometer (LI-COR, Lincoln, NE) with a photosynthetic range (400−700 nm) quantum sensor was used.

Radiochemical Analysis. A Beckman (Fullerton, CA) model LS6000IC liquid scintillation counter was used for quantification of bulk radioactivity in aqueous samples or samples that were combusted. Aqueous samples (100 μL) were dissolved in Scinti Verse (Fisher Scientific) cocktail, and $^{14}CO_2$ evolved from tissue combustion was trapped in RJ Harvey scintillation cocktail. A Packard (Meriden, CT) Radiomatic 525TR flow scintillation analyzer was used with liquid chromatography to quantify labeled RDX, formaldehyde−DNPH, and other products. Ultima-Flo M (Packard) cocktail (3:1 cocktail/eluent) was used.

Tissue Combustion. An RJ Harvey (Hillsdale, NJ) model OX-600 tissue combustion system was used. The temperature was 900 °C, and the nitrogen and oxygen flows were 350 mL/min. RJ Harvey scintillation cocktail was the $^{14}CO_2$ trapping solution, and the sample size was typically 1 g.

RDX in Aqueous Solution. *RDX, N_2O, Nitrite.* A 400 μL aliquot of RDX solution (in acetonitrile) was added to 1.6 mL of deionized (DI) water (704 μM initial RDX) in each of three 12.5 mm × 12.5 mm × 45 mm, 3.5 mL far-UV cells with threaded hole caps (R-3010-T, Spectrocell, Oreland, PA). The cells transmit over 80% of light between 170 and 2200 nm. The cells received 3900 μmol/m^2/s of photon flux from a 1000 W metal-halide lamp (Phyto-Lite I, Hummert International, Earth City, MO) as measured by a photon flux sensor. A photon flux of 3900 μmol/m^2/s is nearly twice that measured on a sunny July day in Iowa (42° N latitude). The lamp spectrum was less continuous than natural sunlight but had strong output in the Soret absorption band (410−460 nm) of chlorophyll *a*. Samples (100 μL) for RDX and UV-active products were taken from one cell and injected into a sealed, 2 mL autosampler vial containing 650 μL of DI water and 750 μL of acetonitrile for HPLC analysis. Samples (100 μL) for nitrite were taken from another cell and analyzed via ion chromatography. Headspace samples (250 μL) for N_2O were taken from the remaining cell and analyzed via GC-ECD. The solution temperature within the cells was 38 °C during the experiment.

$^{15}N^{14}NO$. A 400 μL aliquot of ^{15}N-ring-labeled RDX solution (in acetonitrile) was added to 1.6 mL of DI water (900 μM initial RDX) in a far-UV cell. The cell received 3900 μmol/m^2/s of photon flux, and the solution temperature within the cells was 38 °C. ^{15}N-labeled nitrous oxide ($^{15}N^{14}NO$) was measured by GC/MS.

4-Nitro-2,4-diazabutanal. Clear glass bottles (250 mL) with Mininert caps (13 mm) contained 150 mL of DI water with various forms of RDX. Three reactors contained RDX (41 μM), three contained ^{13}C-RDX (108 μM), three contained ^{15}N-ring-RDX (90 μM), and two contained ^{12}C/^{14}C-RDX (72 uM/1.8 μCi). An additional bottle containing RDX was sampled regularly for RDX disappearance and degradate formation. A final reactor containing RDX was wrapped with aluminum foil for use as a thermal degradation control. The bottles were placed sideways under the metal-halide lamp at a photon flux of 1400 μmol/m^2/s. At the conclusion of the experiment, a 750 μL sample was taken from each reactor and diluted with 750 μL of acetonitrile in a 2 mL crimped vial for LC/MS analysis. Selected ion monitoring analysis methods ranging from *m*/*z* 70 to *m*/*z* 199 at 1 mass unit increments were used.

Formaldehyde. Three clear glass bottles (250 mL) with Mininert caps (13 mm) contained 150 mL of DI water with RDX (72 μM) and 1.8 μCi of ^{14}C-RDX. The bottles were placed sideways under the metal-halide lamp at a photon flux of 1400 μmol/m^2/s. Total radioactivity was determined by sampling (100 μL) using a glass syringe and injecting directly into Scinti Verse cocktail for liquid scintillation analysis (Beckman LS6000IC). At the conclusion of the experiment, 100 mL portions of the reactor solutions were prepared for ^{14}C-formaldehyde analysis using a modified EPA Method 8315A protocol. An acetate buffer solution (5 M) was prepared by adding 40 mL of 5 M acetic acid to 60 mL of 5 M sodium acetate solution. The solution was mixed thoroughly, and the pH was adjusted to 5.0 with HCl or NaOH. A DNPH reagent (3 mg/mL) was prepared by dissolving 428.7 mg of 70% (w/w) DNPH in 100 mL of acetonitrile.

Acetate buffer (4 mL) was added to 100 mL of sample in a 250 mL glass screw cap bottle. The pH was adjusted to 5.0 ± 0.1 with 6 M HCl or 6 M NaOH. DNPH reagent (6 mL) was added; the container was sealed and placed in a heated (40 °C) shaker bath to provide gentle swirling for 1 h. The solution was serially extracted with three 20 mL portions of dichloromethane (DCM). The DCM was removed from the bottom of the bottles with disposable glass pipets and transferred to 100 mL volumetric flasks. The DCM was evaporated completely using a stream of dry nitrogen. The contents of the flask were reconstituted in 5 mL of acetonitrile, and a 750 μL aliquot was removed and diluted with 750 μL of DI water in a 2 mL vial for subsequent flow through radiochemical analysis (Packard 525TR).

RDX in Reed Canary Grass. Hydroponic uptake of RDX in reed canary grass was performed in Erlenmeyer flasks (250 mL) wrapped with aluminum foil. Each of four flasks contained RDX (90 μM), ^{13}C-RDX (90 μM), ^{15}N-ring-RDX (90 μM), or ^{12}C/^{14}C-RDX (90 μM/15 μCi) in 150 mL of 10% Hoagland's solution with a plant. A control flask contained a plant and 150 mL of 10% Hoagland's solution with no RDX. Fluorescent lights were used in a daily cycle of 16 h on (60

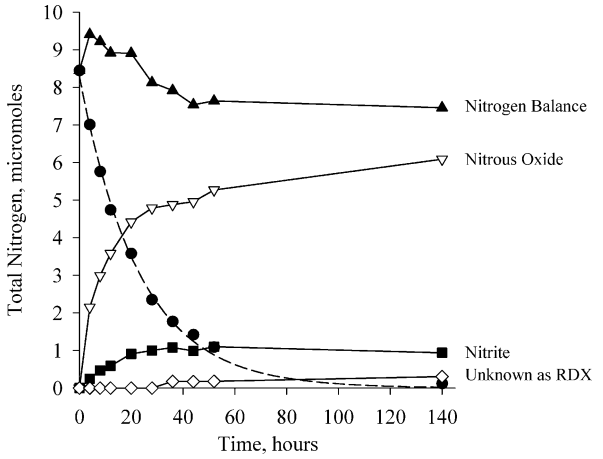

FIGURE 1. Photodegradation of RDX in deionized water. Nitrous oxide and nitrite were detected after 4 h, and an unknown degradate was detected after 36 h. The RDX first-order curve fit (dashed line) revealed a degradation coefficient of 0.046 h^{-1}.

μmol/m^2/s) and 8 h off. Room temperature during the experiment was 22 °C.

After 72 h of RDX uptake, portions of the leaves (0.1−0.3 g) were removed, cut into small sections, and placed inside individual far-UV cells for each RDX isotope tested (two for ^{14}C-RDX). An additional leaf portion from each stable isotope RDX treatment was extracted with acetonitrile and analyzed for total RDX content. A portion of the ^{14}C-RDX-laden leaf tissue (0.2 g) was combusted to determine the initial total radioactivity prior to high-intensity light exposure.

Two thermal (no light) controls (22 and 38 °C) consisted of aluminum foil covered, 4 mL, glass vials containing RDX-laden leaf tissue. The quartz cuvettes received 2000 μmol/m^2/s of photon flux from the metal-halide lamp.

N_2O, 4-Nitro-2,4-diazabutanal. During the experiment, headspace samples (100 μL) for N_2O were taken with a gastight syringe inserted through the septa-lined hole-cap. At the conclusion of the experiment, the leaves were removed from the reactors, finely chopped, and placed in 2 mL of acetonitrile in a 4 mL vial. After 1 h, 1.5 mL of the acetonitrile was transferred to a 2 mL vial for analysis via LC/MS.

Formaldehyde and CO_2. At the conclusion of the experiment, the leaf tissue was combusted to determine the total radioactivity remaining after light exposure. $^{14}CO_2$ production was monitored with time by taking a headspace sample (500 μL) with a gastight syringe, injecting into 10 mL of RJ Harvey scintillation cocktail, and counting with the Beckman LS6000IC. Background samples were collected in a control reactor using the same procedure. Another ^{14}C-RDX-containing reactor was sampled for ^{14}C-formaldehyde using a DNPH cartridge with a disposable needle and a 5 mL plastic syringe. The headspace was evacuated several times using the syringe to pull sample through the cartridge.

Results and Discussion

RDX in Aqueous Solution. *RDX, N_2O, Nitrite.* RDX (8.45 μmol as N initially) was degraded to only 0.077 ± 0.046 μmol within 140 h, following first-order kinetics (0.046 h^{-1}) (Figure 1). After 4 h, 2.15 μmol of nitrous oxide (as N) was detected, and formation continued until reaching 6.1 μmol (72.2% of total N) after 140 h. Nitrite was detected after 4 h and then reached a value of 0.94 μmol (11.1% of total N) after 140 h. Nitrate was not detected. An unknown (presumably 4-nitro-2,4-diazabutanal) compound was detected after 36 h and was reported as RDX-N in Figure 1.

$^{15}N^{14}NO$. Carbon dioxide (*m*/*z* 44) partially coeluted with N_2O, making mass spectral interpretation more difficult. The

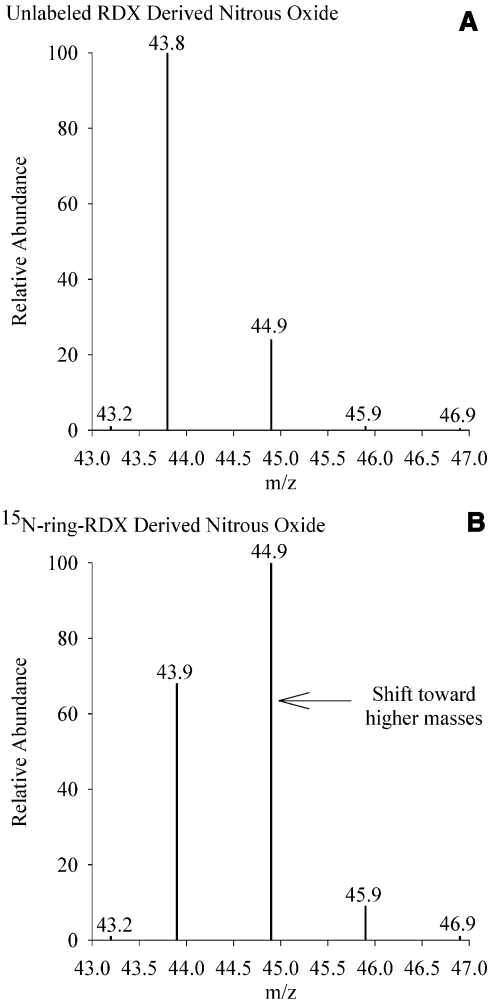

FIGURE 2. (A) The mass spectrum of $^{14}N^{14}NO$ (*m*/*z* 44) derived from unlabeled RDX contained a large signal at *m*/*z* 44 and a less intense signal at *m*/*z* 45. (B) The mass spectrum for nitrous oxide derived from ^{15}N-ring-RDX revealed a shift from *m*/*z* 44 to *m*/*z* 45 indicating $^{15}N^{14}NO$ (*m*/*z* 45) formation from RDX ring cleavage.

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4-Nitro-2,4-diazabutanal, Formaldehyde. The product distribution for RDX, 4-nitro-2,4-diazabutanal, and formaldehyde after 120 h of irradiation was 42%, 32%, and 26%, respectively. The presence of 4-nitro-2,4-diazabutanal was confirmed with LC/MS utilizing stable isotope shift analysis (Figure 3). The compound that eluted at 1.63 min from the RDX reactor had a *m*/*z* of 118 $[M - H]^-$ corresponding to a molecular weight of 119 ($C_2H_5N_3O_3$). The same degradate from the ^{13}C-RDX reactor had a *m*/*z* of 120 $[M - H]^-$ as did the degradate from the ^{15}N-ring-RDX reactor, corresponding to molecular weights of 121 ($^{13}C_2H_5N_3O_3$ and $C_2H_5{}^{14}N^{15}N_2O_3$, respectively).

RDX in Reed Canary Grass. *N_2O, 4-Nitro-2,4-diazabutanal.* Nitrous oxide was produced during high-intensity light exposure, reaching a maximum of 2.35 μmol (74.6%) after 16 h (Figure 4). Only 0.13 μmol (4.1%) and 0.36 μmol (11.4%) of nitrous oxide were produced in the 20 and 38 °C (no light) control reactors, respectively.

The LC/MS data from plant extracts showed an RDX degradate in the RDX extract at *m*/*z* 118 (1.95 min) and one

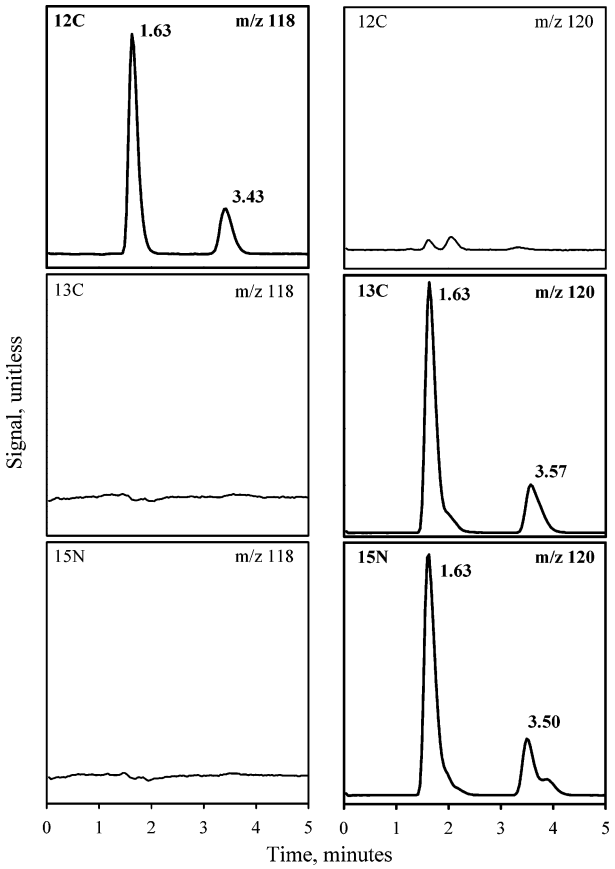

FIGURE 3. Mass spectra (*m/z* 118 and 120) for the end products of aqueous RDX photolysis. The early eluting degradate from the RDX reactor had a *m/z* of 118 $[M - H]^-$ (top left) corresponding to a molecular weight of 119. The same degradate from the ^{13}C-RDX reactor (middle right) had a *m/z* of 120 $[M - H]^-$ as did the degradate from the ^{15}N-ring-RDX reactor (bottom right), corresponding to molecular weights of 121.

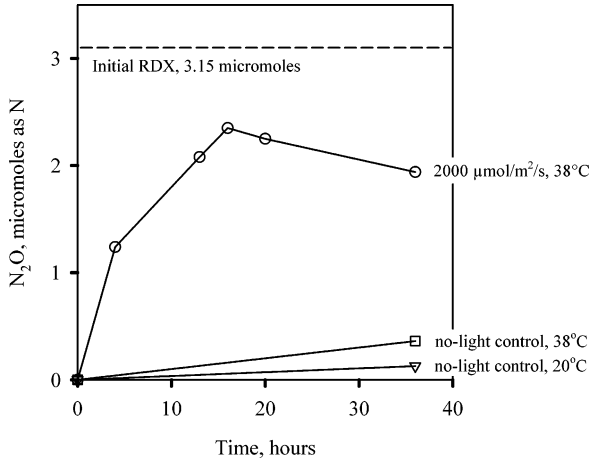

FIGURE 4. N_2O production from excised reed canary grass leaves in quartz cuvettes exposed to simulated sunlight.

peak in each of the ^{13}C-RDX (2.26 min) and ^{15}N-ring-RDX (2.19 min) extracts at *m/z* 120 (Figure 5). Stable isotope mass shifts indicate the degradate contained two RDX-derived carbon atoms and two RDX-derived nitrogen atoms. 4-Nitro-2,4-diazabutanal has a molecular formula of $C_2H_5N_3O_3$ (molecular weight = 119) and has two carbon atoms and two nitrogen atoms in a configuration consistent with partial RDX ring degradation. The deprotonated molecular ion $[M - H]^-$ for 4-nitro-2,4-diazabutanal would have a *m/z* of 118

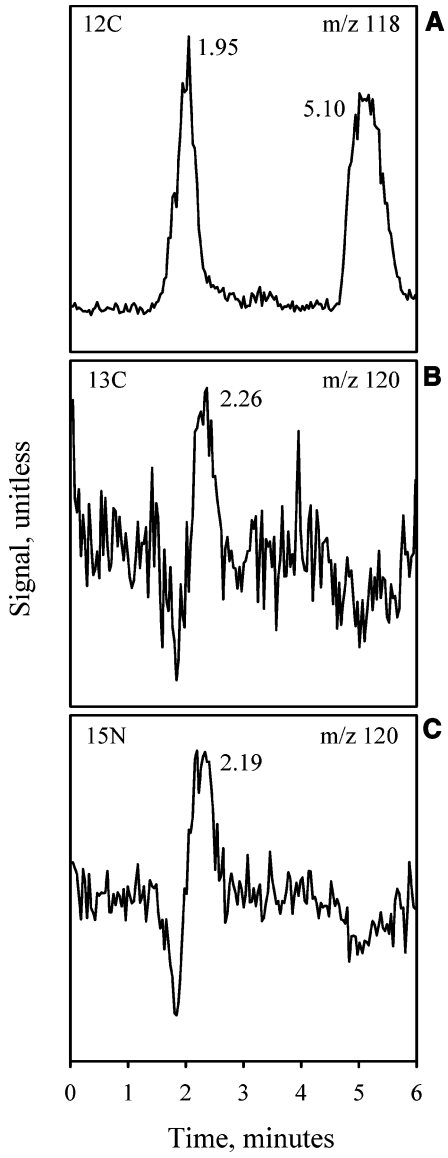

FIGURE 5. Mass spectra of plant extracts after simulated sunlight exposure. Two peaks, identified as 4-nitro-2,4-diazabutanal and RDX, were detected at *m/z* 118 for the RDX exposed leaves (A). One peak at *m/z* 120 was detected in each of the ^{13}C-RDX (B) and ^{15}N-ring-RDX (C) exposed leaves, providing further evidence for 4-nitro-2,4-diazabutanal formation.

for the nonlabeled experiment and a *m/z* of 120 for each of the labeled experiments as supported by the data.

Formaldehyde and CO_2. The initial total leaf radioactivity was 0.30 μCi for a 0.2 g sample. After the leaves were placed in the quartz cuvettes, $^{14}CO_2$ evolution was measured over time but totaled only 1.3% of initial ^{14}C after 55 h and was considered insignificant. Tissue combustion after light exposure showed that 0.23 μCi (77% of initial) of radioactivity remained in the leaf tissue, but not as RDX which was degraded to below detection level (Figure 6). No ^{14}C-formaldehyde was detected in the headspace, possibly due to *S*-formylglutathione formation via a glutathione-dependent formaldehyde dehydrogenase enzyme (*39*, *40*).

By combining RDX photodegradation data from aqueous experiments with those of plant experiments and incorporating the RDX degradation work of other researchers (*21*, *41*), an RDX phytophotolysis pathway (Figure 7) is proposed. 4-Nitro-2,4-diazabutanal and nitrous oxide were detected after exposing RDX-laden leaves to simulated sunlight. Formaldehyde is a likely product, as supported by analogy

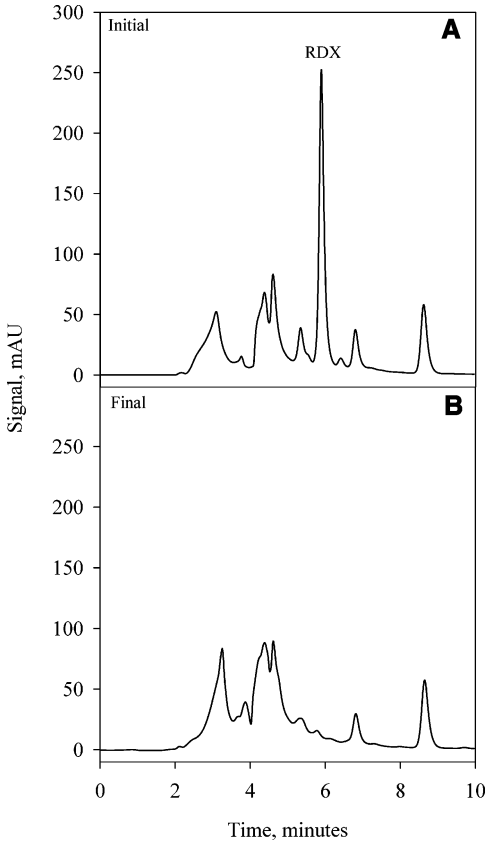

FIGURE 6. Plant extracts (A) prior to simulated sunlight exposure and (B) after exposure showing complete RDX degradation after 55 h.

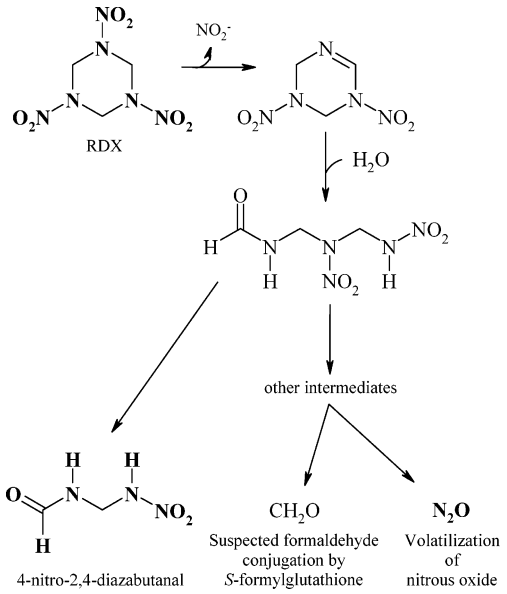

FIGURE 7. Phytophotolysis pathway for RDX degradation in reed canary grass. The compounds shown in bold were detected in reed canary grass, and the compounds shown in regular text have been detected or suggested in work by others (*41*).

to aqueous photolysis results, but was not detected in experiments with reed canary grass.

These results indicate that photolysis via light passage through translucent leaves is a probable mechanism of initial transformation of RDX in phytoremediation of reed canary grass. Following photolytic conversion to N_2O, CH_2O, and 4-nitro-2,4-diazabutanal, plant enzymes such as *S*-formylglutathione may conjugate the formaldehyde resulting in "bound residues" that were observed within the plant tissue in this research. The plant rapidly degraded RDX to smaller products, but the presence and ultimate fate of 4-nitro-2,4-diazabutanal, a potentially toxic product, requires further study. Direct and indirect photolysis can play important roles in the transformation of xenobiotics that have been translocated to shoots and leaves, and the process of phytophotolysis should receive greater attention in phytoremediation applications.

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Literature Cited

(1) Binks, P. R.; Nicklin, S.; Bruce, N. C. *Appl. Environ. Microbiol.* **1995**, *61*, 1318−1322.
(2) Boopathy, R.; Manning, J. F. *Water Environ. Res.* **2000**, *72*, 238−242.
(3) Groom, C. A.; Beaudet, S.; Halasz, A.; Paquet, L.; Hawari, J. *J. Chromatogr., A* **2001**, *909*, 53−60.
(4) Hawari, J. In *Biodegradation of Nitroaromatic Compounds and Explosives*; Spain, J., Hughes, J., Knackmuss, H.-J., Eds.; Lewis Publishers: New York, 2000; pp 277−310.
(5) Hawari, J.; Halasz, A.; Sheremata, T.; Beaudet, S.; Groom, C.; Paquet, L.; Rhofir, C.; Ampleman, G.; Thiboutot, S. *Appl. Environ. Microbiol.* **2000**, *66*, 2652−2657.
(6) Hawari, J.; Shen, C. F.; Guiot, S. R.; Greer, C. W.; Rho, D.; Sunahara, G.; Ampleman, G.; Thiboutot, S. *Water Sci. Technol.* **2000**, *42*, 385−393.
(7) McCormick, N. G.; Cornell, J. H.; Kaplan, A. M. *Appl. Environ. Microbiol.* **1981**, *42*, 817−823.
(8) Sheremata, T. W.; Halasz, A.; Paquet, L.; Thiboutot, S.; Ampleman, G.; Hawari, J. *Environ. Sci. Technol.* **2001**, *35*, 1037−1040.
(9) Sheremata, T. W.; Hawari, J. *Environ. Sci. Technol.* **2000**, *34*, 3384−3388.
(10) Zhao, J. S.; Halasz, A.; Paquet, L.; Beaulieu, C.; Hawari, J. *Appl. Environ. Microbiol.* **2002**, *68*, 5336−5341.
(11) Bhushan, B.; Halasz, A.; Spain, J.; Thiboutot, S.; Ampleman, G.; Hawari, J. *Environ. Sci. Technol.* **2002**, *36*, 3104−3108.
(12) Fournier, D.; Halasz, A.; Spain, J.; Fiurasek, P.; Hawari, J. *Appl. Environ. Microbiol.* **2002**, *68*, 166−172.
(13) Oh, B.-T.; Just, C. L.; Alvarez, P. J. J. *Environ. Sci. Technol.* **2001**, *35*, 4341−4346.
(14) Bier, E. L.; Singh, J.; Li, Z. M.; Comfort, S. D.; Shea, P. J. *Environ. Toxicol. Chem.* **1999**, *18*, 1078−1084.
(15) Bose, P.; Glaze, W.; Maddox, S. *Water Res.* **1998**, *32*, 1005−1018.
(16) Bose, P.; Glaze, W.; Maddox, S. *Water Res.* **1998**, *32*, 997−1004.
(17) Heilmann, H.; Wiesmann, U.; Stenstrom, M. *Environ. Sci. Technol.* **1996**, *30*, 1485.
(18) Botcher, T. R.; Wight, C. A. *J. Phys. Chem.* **1994**, *98*, 5441−5444.
(19) Rauch, F.; Fanelli, A. *J. Phys. Chem.* **1969**, *73*, 1604−1608.
(20) Capellos, C.; Papagiannakopoulos, P.; Liang, Y. L. *Chem. Phys. Lett.* **1989**, *164*, 533−538.
(21) Hawari, J.; Halasz, A.; Groom, C.; Deschamps, S.; Paquet, L.; Beaulieu, C.; Corriveau, A. *Environ. Sci. Technol.* **2002**, *36*, 5117−5123.
(22) Lee, S. J.; Son, H. S.; Lee, H. K.; Zoh, K. D. *Water Sci. Technol.* **2002**, *46*, 139−145.

(23) Spanggord, R.; Mabey, W.; Chou, T.-W.; Lee, S.; Alferness, P.; Tse, D.; Mill, T. *Environmental Fate Studies of HMX, Phase II − Detailed Studies. Contract No. DAMD17-82-C-2100*; U.S. Government Printing Office: Washington, DC, 1983.
(24) Spanggord, R. J.; Mabey, W. R.; Mill, T.; Chou, T.-W.; Smith, J. H.; Lee, S.; Roberts, D. *Environmental Fate Studies on Certain Munitions Wastewater Constituents. Phase IV − Lagoon Model Studies*; SRI International: Menlo Park, CA, 1983.
(25) Best, E. P. H.; Sprecher, S. L.; Larson, S. L.; Fredrickson, H. L.; Bader, D. F. *Chemosphere* **1999**, *39*, 2057−2072.
(26) Bhadra, R.; Wayment, D. G.; Williams, R. K.; Barman, S. N.; Stone, M. B.; Hughes, J. B.; Shanks, J. V. *Chemosphere* **2001**, *44*, 1259−1264.
(27) Harvey, S.; Fellows, R.; Cataldo, D.; Bean, R. *Environ. Toxicol. Chem.* **1991**, *10*, 845−855.
(28) Thompson, P. Phytoremediation of Munitions (RDX, TNT) Waste at the Iowa Army Ammunition Plant With Hybrid Poplar Trees. Ph.D. Thesis, The University of Iowa, Iowa City, IA, 1997.
(29) Thompson, P.; Ramer, L.; Schnoor, J. *Environ. Toxicol. Chem.* **1999**, *18*, 279−284.
(30) Just, C. L.; Schnoor, J. L. *Int. J. Phytorem.* **2000**, *2*, 255−267.
(31) Hannink, N. K.; Rosser, S. J.; Bruce, N. C. *Crit. Rev. Plant Sci.* **2002**, *21*, 511−538.
(32) Medina, V. F.; Larson, S. L.; Agwaramgbo, L.; Perez, W. *Int. J. Phytorem.* **2002**, *4*, 143−156.
(33) Sandermann, H. *Pharmacogenetics* **1994**, *4*, 225−241.
(34) Zhao, X. S.; Hintsa, E. J.; Lee, Y. T. *J. Chem. Phys.* **1988**, *88*, 801−810.
(35) Choi, M.; Kim, H.; Chung, C. *J. Phys. Chem.* **1995**, *99*, 15785−15789.
(36) Zuckermann, H.; Greenblatt, G. D.; Haas, Y. *J. Phys. Chem.* **1987**, *91*, 5159−5161.
(37) Ampleman, G.; Thiboutot, J.; Marois, A.; Hawari, J.; Jones, A.; Rho, D. *J. Labelled Compd. Radiopharm.* **1994**, *36*, 559−577.
(38) Hoagland, D. R.; Arnon, D. I. *Circular* **1950**, *347*, 1−32.
(39) Wippermann, U.; Fliegmann, J.; Bauw, G.; Langebartels, C.; Maier, K.; Sandermann, H. *Planta* **1999**, *208*, 12−18.
(40) Dixon, D. P.; Cummins, I.; Cole, D. J.; Edwards, R. *Curr. Opin. Plant Biol.* **1998**, *1*, 258−266.
(41) Balakrishnan, V. K.; Halasz, A.; Hawari, J. *Environ. Sci. Technol.* **2003**, *37*, 1838−1843.

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