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Toxicity and Uptake of Nitroguanidine in Plants

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During the manufacture of the munition nitroguanidine (NQ), $(NH_2)_2C{=}N{-}NO_2$, wastewater is contaminated by NQ and related by-products (Burrows et al 1984). Land application of the NQ-contaminated wastewater was initiated after discussions with the Kansas Department of Health and the Environment and the US Environmental Protection Agency. This treatment method has been a cost-effective and convenient means of disposal (Nickelson 1982). Knowledge of the toxicity and environmental fate of NQ will facilitate safe disposal of the wastewater.

The environmental fate of NQ is related to plant uptake and metabolism as well as metabolism by plant consumers and decomposers. Although it is water soluble, NQ is not present in ground water samples taken near the application sites. Kaplan et al (1982) reported that anaerobic sludge organisms slowly converted NQ to nitrosoguanidine but that activated sludge cultures did not biodegrade NQ under aerobic conditions. Microorganisms which aerobically degraded 1,3-dinitrobenzene did not degrade NQ (Dr. Wayne Mitchell, US Army Biomedical Research and Development Lab, Fort Detrick, Frederick, MD, unpublished). Ho et al (1988) recovered NQ quantitatively and unchanged in the urine of rats which were administered either oral or intravenous doses of NQ.

Toxicity of NQ in vertebrates has been found to be very low. Studies showed that NQ was non-toxic to four fish species after 96 h exposure to 20 mM (van der Schalie 1982) and the lethal dose in rats was greater than 5.0 g kg^{-1} (Hiatt et al 1985).

Land application of NQ-contaminated wastewater will result in NQ penetration into the root zone where the potential for absorption exists. However, virtually nothing is known of the uptake and metabolism of NQ in plants. The objective of this study was to test the toxicity of and to characterize the uptake of NQ in plants.

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MATERIALS AND METHODS

Toxicity of NQ was evaluated by inhibition of growth, leaf chlorosis, and nutrient (Mg, Ca, and K) analysis of plants exposed to NQ. Preliminary studies provided data (unpublished) concerning the expected effects of selected NQ concentrations and a basis for the studies reported below. The solubility of NQ in water is 4.4 g L^{-1} (42 mM) at 25 C and its vapor pressure is extremely low. Both NQ solid and solutions were handled using normal laboratory procedures except that disposable gloves were worn.

Initially, we tested the effects of NQ on hydroponically grown soybean (Glycine max L. Merr., cv. Williams). Individual one-week-old seedlings were transferred to 850-mL jars of aerated hydroponic solution (Triplett et al 1980). Plants were raised in a growth room with a day/night temperature of 25/20 C, a 14-hr photoperiod, and with a visible light intensity of 400 μmol (quanta) m^{-2} s^{-1}.

Fourteen days after germination, at the V1 stage of growth (Fehr and Caviness, 1977), the hydroponic solutions were adjusted to 0, 2, or 4 mM NQ. Leaf chlorophyll concentration (Inskeep and Bloom, 1985) was determined 25 days after germination (V5) and plants were harvested, oven dried at 60 C for two days, and weighed. Ground leaf samples (30 mesh) were digested with nitric acid and perchloric acid and cation (Ca, Mg, and K) concentration determined using atomic absorption spectrometry. Four replications (one jar each) were used.

The effect of NQ on plants grown in soil was tested on soybean and two grass species. Three genotypes each of soybean (cvs. Williams, Washington, and Wayne), tall fescue (Festuca arundinacea Schreb., cvs. KY 31, Mozark, Martin), and smooth bromegrass (Bromus inermis Leyss., cvs. Achenbach, Blair, and Beacon) seed were planted in 600-mL pots containing a soil mixture. These species are commonly grown agriculturally near at least one NQ production site. Soil mixture water holding capacity was 100 mL pot^{-1}. Ten days after germination, grasses were thinned to two per pot and soybeans to one per pot. Plants were raised in the greenhouse with a day/night temperature of 25/20 C and watered weekly with 1/4 strength Hoagland's solution (Hoagland and Arnon 1950).

Twenty-three-day-old soybean and 30-day-old tall fescue and smooth bromegrass plants were treated with 100 mL of either 0, 2, 4, 6, or 8 mM NQ solutions (all prepared with 1/4 Hoagland's solution) by applying the solution directly to the soil. Solutions of NQ were supplied on alternate days for a total of 5 applications. Leaf chlorophyll concentration (tall fescue and smooth bromegrass only) was determined two weeks after the initial treatment. Plants were then harvested and tissue separated as follows: (1) soybeans: leaf blades, stems including petioles, and washed roots, (2) grasses: leaves plus culms, and washed roots. Samples were oven-dried at 60 C for two days and weighed. Ground leaf samples

from Kentucky 31 tall fescue and Achenbach smooth bromegrass were analyzed for Ca, Mg, and K concentrations as described previously. Five replications, of one pot each, were used.

Comparisons of the uptake and metabolism of NQ between soybean and tall fescue and between hydroponic and soil culture were conducted. Growth room conditions, soybean (cv. Williams) culture (both soil and hydroponic) and tall fescue culture (soil) were described previously. For tall fescue (cv. Kentucky 31) hydroponic culture a pair of one week old seedlings were transferred to 850 mL of solution described by Edwards and Pedersen (1986). After 21 days (soybean, V4 stage) and 30 days (tall fescue) the soil or hydroponic solutions were adjusted to either 6.5×10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, or 5×10^{-3} M ^{14}C-NQ solution. Radioactive NQ (18.3 mCi mmol^{-1}) was obtained from NEN Research Products, E. I. du Pont de Nemours Co., Inc. Each pot or jar received 3.70×10^{4} Bq ^{14}C-NQ. Soil grown plants received a single 100-mL application of the NQ treatment as a root drench. Hydroponic and soil media without plants were included as controls. After 96 hr, aliquots (2.0 ml) were obtained from hydroponic solution and the quantity of ^{14}C determined using scintillation spectrometry. The quantity of ^{14}C from aliquots of soil (200 mg air dried) was determined by both scintillation spectrometry after methanol extraction and sample combustion (Ho et al 1988). Plants were harvested, broken into parts described earlier, freeze-dried, and weighed. Ground samples (200 mg aliquots) were oxidized and the quantity of ^{14}C determined. Metabolism and degradation compounds of NQ were examined after extraction with methanol:water (80:20, v/v) by thin layer chromatography using butanol, ethanol, and water (66:17:17, v/v/v) on cellulose plates. Three replications were used.

RESULTS AND DISCUSSION

Soybean dry matter and concentrations of leaf chlorophyll and Mg were reduced by 2 and 4 mM NQ in hydroponic culture (Table 1). The NQ-induced chlorosis was distributed throughout the leaf but was more prominent at the leaf margins. Calcium concentration was reduced by 4 mM NQ but not by 2 mM NQ and K concentration was increased by 2 mM.

Table 1. Effect of NQ in hydroponic solution on dry matter and concentrations of chlorophyll, Ca, Mg, and K of soybean leaves.

NQ Concentration	Dry Matter	Chlorophyll	Ca	Mg	K
mM	g plant^{-1}	μg cm^{-2}	-------	g kg^{-1}	------
0	2.92	53.7	14.9	7.0	41.7
2	1.94	44.1	13.9	6.2	47.5
4	1.18	30.9	12.1	6.0	44.6
$LSD_{0.05}$	0.41	5.5	1.7	0.6	5.4

In soil all genotypes within a given species responded similarly to NQ treatments and all data within a species were averaged across genotypes. Soybeans produced less dry matter (Fig. 1) as the concentration of NQ in the solutions applied to the soil increased from 0 to 8 mM NQ. The average dry matter of untreated tall fescue plants was 0.62 g plant^{-1}, and was significantly reduced only by the 8 mM NQ (0.51 g plant^{-1}) (Fig. 1). Dry matter accumulation by smooth bromegrass was unaffected by NQ treatment. Calcium concentration of both tall fescue and smooth bromegrass leaves was reduced by 100 ml applications of 4 and 8 mM NQ to the soil (Table 2). There was no significant effect of NQ on leaf chlorophyll, Mg, or K concentrations of the grasses.

Table 2. Effect of five 100 mL soil drenches containing either 0, 4, or 8 mM NQ on chlorophyll, Ca, Mg, and K concentrations of tall fescue and smooth bromegrass leaves.

Species	NQ concentration	Chlorophyll	Ca	Mg	K
	mM	μg cm^{-2}	----	g kg^{-1}	----
Tall fescue	0	81.3	2.8	3.2	48
	4	-	2.6	3.3	50
	8	80.8	2.4	2.9	49
Smooth bromegrass	0	70.3	3.1	2.6	44
	4	-	2.3	2.4	45
	8	65.9	2.0	2.4	48
$LSD_{0.05}$		NS	0.05	NS	NS

Characteristics of NQ uptake and accumulation were determined by exposing roots of intact plants to selected NQ concentrations. The quantity of ^{14}C recovered from oxidation of plant material was converted to NQ-equivalents since radioactivity of leaf extracts (R_f=0.41) co-chromatographed with standard NQ (R_f=0.43). Concentration of NQ-equivalents in roots and leaves of both species was significantly greater from plants grown in hydroponic media than those grown in soil (Table 3). Kaplan et al (1982) suggested that both physical and biological reactions with NQ in soil could contribute to its breakdown and loss. We obtained incomplete and variable recovery of ^{14}C-NQ from air-dried soil 96 hr after ^{14}C-NQ solutions were added to soil in the absence of plants (39% and 70% using methanol extraction and combustion, respectively). In contrast to recovery from soil, we obtained complete recovery of ^{14}C-NQ after 96 hr from plant-free hydroponic media. The apparent loss of NQ from the soil could have reduced the NQ concentration in the soil solution and contributed to the lower tissue NQ concentration of soil grown plants compared to that of hydroponically grown plants.

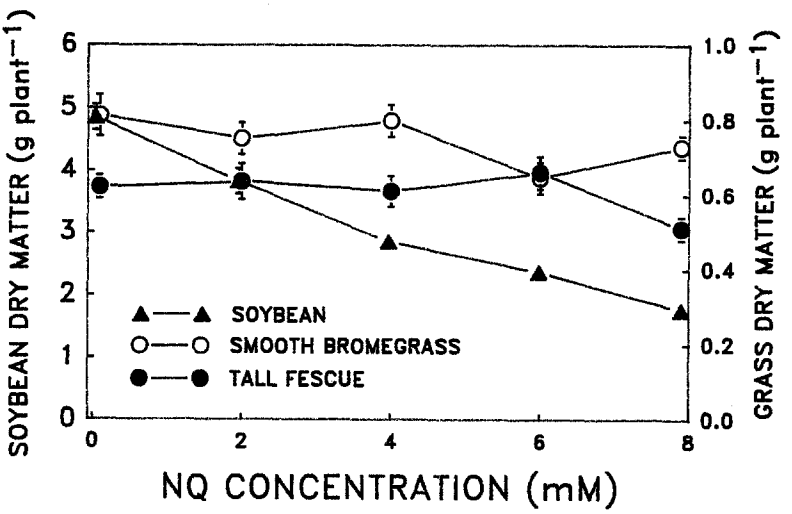

Figure 1. Effect of five 100-ml applications of 0 to 8 mM NQ on dry matter accumulation of three plant species.

Table 3. Concentration of NQ in soybean and fescue plant parts after exposure to 5 x 10^{-3}, 10^{-3}, or 10^{-6} M NQ for 96 hr. Concentrations are based upon ^{14}C-recovered.

Media NQ Concentration	Culture	Species	Tissue NQ Concentration Roots	Stems	Leaves
			-- μmol g^{-1} dry matter ---		
5 x 10^{-3} M	Hydroponic	Tall fescue	29.8	-	78.4
		Soybean	44.3*	9.8	195.0*
	Soil	Tall fescue	8.1	-	23.3
		Soybean	7.2	3.6	66.6*
1 x 10^{-3} M	Hydroponic	Tall fescue	6.4	-	17.7
		Soybean	8.7	2.8	40.3*
	Soil	Tall fescue	1.8	-	4.0
		Soybean	1.7	0.7	11.9*
			-- nmol g^{-1} dry matter ---		
1 x 10^{-6} M	Hydroponic	Tall fescue	6.01	-	16.3
		Soybean	7.95	2.52	41.6*
	Soil	Tall fescue	1.21	-	3.41
		Soybean	1.21	0.548	10.20*

* indicates that concentrations were significantly ($p<0.05$) different between species.

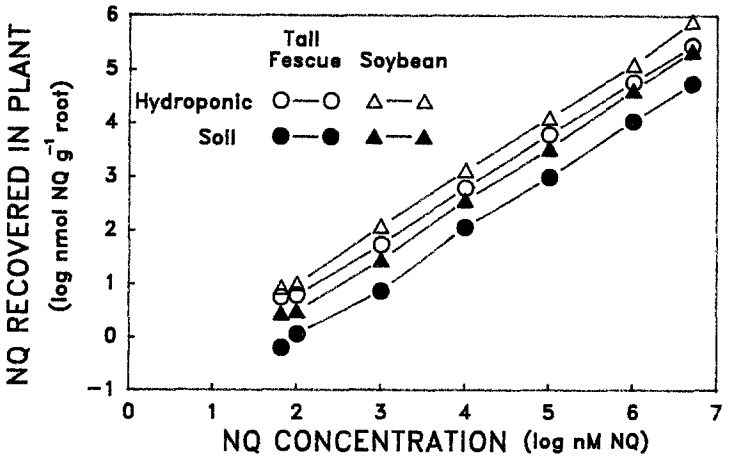

Figure 2. Effect of NQ concentration on NQ equivalents recovered in soybean and tall fescue.

Table 4. The quantity of NQ depleted from solution of hydroponically grown plants, the quantity recovered in the entire plant, apparent loss of ^{14}C-NQ and ratio of NQ recovered to NQ depleted from the plant-culture jar system after 96 hr exposure to hydroponic solutions containing 5 x 10^{-3}, 10^{-4}, or 10^{-6} M NQ.

Solution NQ Concentration	Species	NQ Depleted	NQ Recovered	NQ lost	Recovered: depleted
			µmol		
5 x 10^{-3} M	Tall fescue	134.0	87.4	46.1	0.65
	Soybean	350.0	278.0	72.0	0.79*
1 x 10^{-3} M	Tall fescue	39.3	19.8	19.5	0.50
	Soybean	98.4	71.7	26.7	0.73*
			nmol		
1 x 10^{-6} M	Tall fescue	25.5	19.0	6.5	0.74
	Soybean	97.2	78.4	18.7	0.80

* indicates the recovered:depleted ratio was significantly ($p<0.05$) different between species

The uptake of NQ from both media was passive for both tall fescue and soybean as demonstrated by the linear relationship between total NQ recovered g^{-1} root and external NQ concentration (Fig. 2). The slopes of the lines were not significantly different between treatments. However, the intercepts were significantly higher for hydroponic culture than for soil.

The physiological events associated with the differential toxicity between soybean and tall fescue were not readily apparent. Both species exhibited an NQ-induced decrease in leaf Ca concentration. Soybean depleted more NQ from hydroponic solution (Table 4), accumulated more NQ per g root biomass (Fig. 2), and had greater leaf NQ concentrations than did tall fescue (Table 3). Despite these observations, there is evidence that the greater toxicity of NQ to soybean as compared to tall fescue is primarily due to soybean's greater sensitivity to NQ and not due its greater uptake and accumulation of NQ. Soybean leaves were chlorotic with foliar NQ concentrations of 40.3 μmol g^{-1} whereas tall fescue leaves remained green with NQ concentrations of 78.4 μmol g^{-1} (Table 3). Therefore, it is apparent that the differential toxicity between species is due to differential sensitivity and not due to soybean's greater accumulation and greater leaf NQ concentrations exhibited at each NQ concentration.

The greater uptake and leaf NQ concentration in soybean compared to tall fescue suggest that the roots of these two species differ in their permeability to NQ. However, factors such as differences in root absorptive area per g of root could also be involved. The data also suggest that acropetal transport of NQ may be greater in soybean than in tall fescue.

Since extracts of radiolabeled leaves and roots did not indicate the presence of any metabolite in either species, the biochemical events related to the differential toxicity between species also remain unknown. Despite the apparent absence of NQ metabolism, total plant and hydroponic media ^{14}C-NQ did not account for all ^{14}C initially applied. This suggests that a plant mediated loss of ^{14}C-NQ (or derived but undetected metabolite) occurred from both species (Table 4). The ratio of NQ-equivalents recovered from plant tissue to NQ depleted from ^{14}C-treated hydroponic media averaged 0.77 and 0.63 for soybean and tall fescue, respectively, and was significantly different between species. This loss of C from NQ suggests transformation of NQ into volatile compounds by the plant. The greater ratio of recovered:depleted exhibited by soybean indicates that the loss occurs to a lesser extent in soybean than in tall fescue. Whether this process of ^{14}C-release is the basis for differential toxicity between species is a potential focus for future investigations.

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