Effect of Nitrogen, Sodium, and Potassium on Nitrate and Oxalate Concentration in Kikuyugrass[1]

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Abstract. Kikuyugrass was analyzed for nitrate (expressed as KNO_3) and soluble oxalate concentration after it was grown for 8 wk in nutrient solution supplemented with KCl, NaCl, or NH_4NO_3; fertilized in the greenhouse with urea and KNO_3 at 112 kg N ha^{-1}; and fertilized in the field in Hawaii and Panama with urea at 56 and 112 kg N ha^{-1}. Both treated and untreated kikuyugrass grown in nutrient solution contained toxic levels of nitrates and soluble oxalates. Plants treated with urea in the greenhouse contained 0.4% nitrate (nontoxic) 72 h after treatment whereas those treated with KNO_3 contained 2.36% nitrate (highly toxic). Fertilization of kikuyugrass with urea in Panama and Hawaii did not significantly affect soluble oxalate concentration, but nitrate concentration increased to potentially lethal levels (over 1.5% as KNO_3, dry wt) in plants from Hawaii. **Nomenclature:** Kikuyugrass, *Pennisetum clandestinum* Hochst. ex Chiov. #[3] PESCL.
Additional index words: Fertilizer, KCl, KNO_3, NaCl, poisonous plant, urea.

INTRODUCTION

Kikuyugrass is a native of eastern Africa that has been introduced and cultivated as a pasture grass in Australia, New Zealand, Panama, Hawaii, and other tropical and subtropical areas.

Although kikuyugrass can be grazed safely under most conditions, the plant has been shown to accumulate toxic concentrations of nitrate and soluble oxalate. Poisoning has been reported in swine (*Sus scrofa*) (2), cattle (*Bos tauras*) (7, 8), horses (*Equus caballus*) (9), and sheep (*Ovis aries*) (6).

Three specific syndromes associated with kikuyugrass poisoning have been described. Nitrate poisoning has been reported in cattle and swine that grazed kikuyugrass pastures (2). Chronic oxalate intoxication has been responsible for osteódystrophia fibrosa in horses (3). This condition, commonly called bighead, has been observed in horses that grazed kikuyugrass with as little as 0.3% soluble oxalate (9). Bighead and emaciation have been observed among horses on the experimental ranch in Panama and on the Island of Hawaii. At least five horses in Hawaii died from oxalate poisoning after grazing kikuyugrass pastures for several months. A third syndrome associated with kikuyugrass poisoning involves inflammation and necrosis of the alimentary tract. Called "kikuyu poisoning," it is reported chiefly from New Zealand and primarily affects cattle (2). The cause of this syndrome has not been determined.

The accumulation of toxic levels of soluble oxalate and nitrate in some plants often depends upon the availability of Na and K to form oxalate salts and of N to form nitrate. Addition of NaCl to nutrient cultures significantly increased soluble oxalate levels in halogeton [*Halogeton glomeratus* (Stephen ex Bieb.) C.A. Mey.] # HALGL (10). Fertilization with nitrogen fertilizers may increase nitrate levels in some plants. Plants in which toxic levels of nitrate have been measured have been listed by Kingsbury (5). The effect of nitrogen fertilizers and sodium and potassium salts on accumulation of soluble oxalate and nitrate in kikuyugrass was unknown.

This study was conducted to determine the effect of potassium chloride, sodium chloride and ammonium nitrate added to nutrient solution, and the effect of urea and potassium nitrate added to soil, on the soluble oxalate and nitrate concentration in kikuyugrass foliage.

MATERIALS AND METHODS

Nutrient solution studies–greenhouse. Seeds of kikuyugrass were obtained from the Southern Regional Plant Introduction Station, Experiment, GA. The seeds were germinated in vermiculite and soil (3:1) and the

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[3]Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Revised 1989. Available from WSSA, 309 W. Clark St., Champaign, IL 61820.

seedlings were grown until 3 cm tall. The seedlings were then transferred to 4-L polyethylene pots that contained Hoagland's nutrient solution (4). An iron chelate, HEDTA, was used as a source of iron because this form is used more effectively by monocots. Two plants were inserted through the lid of each pot. The pots were covered with aluminum foil to exclude light and placed on a greenhouse bench. Aeration was supplied at 34 kPa through polyethylene tubes inserted in the lids. Twenty plants were used per treatment (10 pots × 2 plants) and arranged in a completely randomized design with six treatments, including controls, and four replications. The experiment was conducted in the greenhouse in Logan, Utah.

The plants were grown for 2 wk, then solutions were replaced and supplemented with 0.001 M and 0.01 M KCl, 0.001 M and 0.01 M NaCl and 100 meq of nitrogen as NH_4NO_3. One set of 20 plants was used as the control. Nutrient solutions and treatments were replaced every 2 wk for 8 wk. No supplemental light was used. Plants were harvested 8 wk after treatments began by clipping the foliage 2 cm above the crown. The foliage was washed under running water to remove external K, Na, and N salts and dried in a forced-air oven at 50 C for 24 h. The 20 plants from each treatment were randomly divided into four sets of five plants each, and were ground, and analyzed for percent soluble oxalate by the method of Dye (1). Percent nitrate (as KNO_3) was determined by the chromotropic acid method at the Soils Testing Laboratory at Utah State University. The experiment was repeated. The data in Table 1 are an average of two experiments.

Data were analyzed by analysis of variance appropriate for a completely randomized design. Differences among means for these and subsequent experiments were determined by Duncan's Multiple Range Test.

Fertilizer treatments–greenhouse. Kikuyugrass was germinated in April in vermiculite and soil (3:1), then transplanted to 15-cm diam styrofoam pots in vermiculite, peat, and loam soil (1:1:5). The plants were grown on a greenhouse bench without supplemental light or fertilizer. At 10 wk the plants were treated with urea and KNO_3. These compounds were dissolved in distilled water and applied at 112 kg N ha^{-1} in 100 ml of solution. An additional 100 ml of distilled water were added to move the compounds into the root zone. Thirty-two plants were used for each of two treatments and arranged in a completely randomized design. The four plants used per replication were pooled for analysis. All foliage was harvested from four each KNO_3- and urea-treated plants selected at random at 0, 4, 8, 16, 24, 32, 48, and 72 h after treatment. Foliage was clipped 2 cm above the soil surface, washed thoroughly in distilled water to remove any external urea or nitrate salts, dried in a forced-air oven at 50 C, then ground to pass a 40-mesh cm^{-2} screen. The plants were analyzed for soluble oxalate and nitrate. The experiment was repeated and data from the two experiments were averaged.

Fertilizer treatments–field. Experimental plots were established on kikuyugrass pastures in Cerro Punta, Chiriqui, Republic of Panama, and at the Mealani Research Station, University of Hawaii, Kamuela, Island of Hawaii. The grass was in vegetative growth when treated and harvested. Soils in the experimental area in Panama consisted of 77% sand, 17.5% silt, and 5.5% clay, with a pH of 6.4. Depth of topsoil was 0 to 60 cm; depth of subsoil was 60 to 120 cm. Soils in Hawaii were Maile Silt Loam dark reddish brown to dark brown, surface layer to 40 cm, with subsoil 40 cm to 1.2 m, and neutral to slightly acid. The experimental area in Panama had not been fertilized previously whereas the treatment site in Hawaii had been fertilized with 61 kg N ha^{-1} as urea on Mar. 6, 1987.

The 6.1 by 6.1 m plots were arranged in three randomized block designs with three treatments, including an untreated plot. Urea was weighed, then broadcast on individual plots at 56 and 112 kg N ha^{-1} on Nov. 20, 1987 in Hawaii and on Mar. 25, 1988 in Panama. Approximately 20 to 25 g (dry wt) of foliage was clipped at random from each plot on the day of treatment and weekly for 7 wk thereafter. The grass was dried and sent to the Poisonous Plant Research Laboratory at Logan, UT, for nitrate and soluble oxalate analysis. All kikuyugrass samples, upon receipt from Panama and Hawaii, were stored in sealed plastic bags at 0 C until analysis.

RESULTS AND DISCUSSION

Nutrient solution studies–greenhouse. Plants that contain more than 1.5% nitrate, expressed as KNO_3, on a dry weight basis, are considered potentially lethal to ruminants (2). The nitrate level in kikuyugrass foliage from all treatments and the controls exceeded 1.5% (Table 1). Only the nitrate levels in plants grown in solutions that contained 0.01 M KCl were significantly lower in nitrate than controls, but even this level exceeded the toxic threshold and would not have been significantly less toxic to ruminants. The addition of NH_4NO_3 did not significantly increase the nitrate concentration, so it is unlikely that nitrate would exceed 2

Table 1. Effect of N, K, and Na on nitrate and soluble oxalate concentration in kikuyugrass grown in hydroponics in the greenhouse.

Treatment	KNO_3	Soluble oxalates
	%	
Control	2.0 bc[a]	3.1 c
0.001 M KCl	1.9 b	3.6 d
0.01 M KCl	1.6 a	2.6 a
0.001 M NaCl	2.0 bc	2.8 ab
0.01 M NaCl	2.0 bc	3.1 c
100 meg N as NH_4NO_3	2.2 c	2.9 bc

[a]Numbers followed by the same letter within a column do not differ significantly at the 0.05 level by Duncan's Multiple Range Test.

to 3% under optimum field conditions. Nevertheless, when nitrogen, particularly in the form of nitrate, was not limiting, the plants accumulated enough nitrate to be toxic.

The concentration of soluble oxalate was slightly higher than that of controls in plants treated with 0.001 M KCl and lower in plants treated with 0.01 M KCl and 0.001 M NaCl (Table 1). The 0.01 M concentration of KCl depressed both nitrate and soluble oxalate levels. The level of soluble oxalate in all treatments and the controls was far higher than the 0.3% to 0.4% in kikuyugrass that causes bighead in the field. These high levels of soluble oxalate have not been reported in kikuyugrass under field conditions and they undoubtedly resulted from growing the plants with an optimum combination of water, aeration, and essential nutrients. These data indicate that kikuyugrass has the potential to accumulate up to 3% soluble oxalates (dry wt).

Fertilizer treatments–greenhouse. Soluble oxalate concentration in urea and KNO_3-treated plants fluctuated during the test period and both were slightly lower 72 h after treatment (Table 2). Minor differences were found in soluble oxalate concentration between plants treated with urea and KNO_3. The very small differences in oxalate levels between treated and untreated plants were unimportant regarding toxicity of the plants to grazing animals.

The form of nitrogen applied markedly affected nitrate accumulation. The nitrate concentration in plants treated with KNO_3 increased significantly at each collection except at 32 h. Nitrate concentration reached the toxic level at 8 h and the potentially lethal level at 24 h. At 48 h, the foliage contained the same nitrate concentration as controls in the nutrient solution experiment. The nitrate concentration in the urea-treated plants was significantly higher only 72 h after treatment, but the concentration of 0.4% was still below the toxic level of 0.5%.

Fertilizer treatments–field. Except for one collection

Table 2. Nitrate and soluble oxalate concentration in kikuyugrass grown in pots in the greenhouse after fertilization with urea and potassium nitrate at 112 kg N ha^{-1}.

Hours after treatment	Nitrate		Soluble oxalate	
	Urea	KNO_3	Urea	KNO_3
	%		%	
0	0.03 a[a]	0.03 a	0.27 d	0.27 c
4	0.03 a	0.26 b*[b]	0.18 a	0.30 d*
8	0.05 a	0.82 c*	0.18 a	0.29 c*
16	0.04 a	1.36 d*	0.22 bc	0.22 b
24	0.05 a	1.84 e*	0.22 bc	0.29 c*
32	0.07 a	1.72 e*	0.21 b	0.15 a*
48	0.12 a	2.08 f*	0.24 c	0.23 b
72	0.40 b	2.36 g*	0.24 c	0.23 b

[a]Numbers followed by the same letter within a column do not differ significantly at the 0.05 level by Duncan's Multiple Range Test.

[b]Asterisk indicates that means for treatment for a fixed time between columns of a given row are significantly different at the 0.05 level.

of kikuyugrass in Panama, fertilization with urea produced no significant differences in soluble oxalate concentration between treated and untreated plants in Panama and Hawaii (Table 3).

The nitrate concentration in kikuyugrass from Panama peaked at 0.44% 3 wk after treatment, then fell to nonsignificant levels 2 wk later, whereas the nitrate concentration in control plants in Hawaii was at toxic levels throughout the collection period (Table 4). Except for the fourth and fifth collections, nitrate levels were higher in kikuyugrass treated with urea at 0.56 kg N ha^{-1} compared with treatments with 1.12 kg N ha^{-1}. Except for the fifth collection of plants treated with 56 kg N ha^{-1}, nitrate concentration was significantly higher than that of controls and remained in the potentially lethal range for 7 wk after treatment.

Precipitation may have influenced nitrogen incorporation into the soil and oxidation of the urea to nitrate. The average annual precipitation for the test area in Panama is 2100 mm. During the test period only 72 mm were recorded. Of this, 24 mm were recorded the 2nd week and 43 mm the 6th week. Precipitation in Hawaii averaged 234 mm, 185 mm, and 271 mm, in November, December, and January, respectively. Fertilization of the Hawaii test site 8 mo previously provided a soil sufficiently high in available nitrogen to produce potentially toxic plants without additional urea. Addition of more urea, plus generous precipitation which carried the urea through the permeable volcanic soil to the root zone, may have significantly increased the nitrate concentration in the kikuyugrass.

The much lower nitrate concentration found in kikuyugrass in Panama, compared to Hawaii, may be partially attributed to lack of rainfall which prevented

Table 3. Mean percent soluble oxalate concentration in kikuyugrass sampled from field plots in Panama and Hawaii after fertilization with urea.

			Soluble oxalate							
			Week of collection							
Site	Treatment	Rate	0[a]	1	2	3	4	5	6	7
		kg N ha^{-1}	%							
Panama	Control	0	0.5 a[b]	0.6 a	0.5 a	0.4 a	0.5 a	0.4 a	0.4 a	0.5 a
	Urea	56	0.5 a	0.5 a	0.4 a	0.5 a	0.5 a	0.4 a	0.5 a	0.4 a
	Urea	112	0.5 a	0.5 a	0.5 a	0.6 a	0.8 b	0.2 a	0.4 a	0.3 a
Hawaii	Control	0	0.4 a	0.4 a	0.3 a	0.3 a	0.3 a	0.3 a	0.3 a	0.2 a
	Urea	56	0.3 a	0.3 a	0.3 a	0.3 a	0.3 a	0.2 a	0.3 a	0.3 a
	Urea	117	0.3 a	0.4 a	0.3 a	0.3 a	0.3 a	0.4 a	0.4 a	0.4 a

[a]Dates of treatment were November 20, 1987 and March 25, 1988 in Hawaii and Panama, respectively.

[b]Numbers followed by the same letter within a column for different sites do not differ significantly at the 0.05 level by Duncan's Multiple Range Test.

Table 4. Mean percent nitrate concentration (as KNO_3) in kikuyugrass sampled from field plots in Panama and Hawaii after fertilization with urea.

			KNO_3							
			Week of collection							
Site	Treatment	Rate	0[a]	1	2	3	4	5	6	7
		kg N ha^{-1}	%							
Panama	Control	0	0.11 a[b]	0.15 b	0.12 a	0.07 a	0.03 a	0.07 a	0.02 a	0.03 a
	Urea	56	0.09 a	0.10 ab	0.08 a	0.08 a	0.03 a	0.05 a	0.02 a	0.02 a
	Urea	112	0.09 a	0.06 a	0.12 a	0.44 b	0.10 b	0.05 a	0.03 a	0.03 a
Hawaii	Control	0	0.84 a	0.83 a	0.72 a	1.11 a	1.03 a	1.02 a	1.02 a	1.04 a
	Urea	56	0.91 a	2.14 c	2.12 c	1.64 b	1.43 a	1.62 b	1.54 b	1.61 b
	Urea	112	0.83 a	1.52 b	1.59 b	1.58 b	1.44 a	1.32 a	1.29 a	1.02 a

[a]Dates of treatment were November 20, 1987 and March 25, 1988 in Hawaii and Panama, respectively.

[b]Numbers followed by the same letter within a column for different sites do not differ significantly at the 0.05 level by Duncan's Multiple Range Test.

adequate incorporation of the urea into the soil and its subsequent oxidation to nitrate.

Kikuyugrass may rapidly accumulate 2% to 2.5% nitrate, a potentially lethal concentration for livestock, if fertilized with KNO_3. Urea may increase the concentration of nitrate to toxic or potentially lethal levels if soil nitrate levels are already high or environmental conditions are conducive to oxidation of ammonia to nitrate. Urea may also result in nitrate poisoning if applied in large quantities as manure, or if unusual concentrations of animal excreta accumulate in corrals or near ponds where animals congregate. Nitrate poisoning occurred in cattle and pigs in Queensland, Australia, after the animals grazed kikuyugrass fertilized with large amounts of manure (2).

The data indicate that fertilizing soil-rooted kikuyugrass with urea or nitrate nitrogen does not affect the concentration of soluble oxalate in the foliage. However, plants grown in nutrient solution may accumulate 3% soluble oxalate which is 6 to 10 times higher than concentrations observed in the field. Supplementing the nutrition of kikuyugrass with KCl and NaCl did not affect soluble oxalate concentration. This species, therefore, does not use K or Na to form unusually high concentrations of oxalate salts.

LITERATURE CITED

1. Dye, W. B. 1956. Chemical studies on *Halogeton glomeratus*. Weeds 4:55–60.
2. Everist, S. L. 1981. Gramineae (Poaceae). p. 338–340 *in* S. L. Everist, Poisonous Plants of Australia. Angus and Robertson, Publ., Sydney, Australia.
3. Groenendyk, S., and A. A. Seawright. 1974. Osteodystrophia fibrosa in horses grazing *Setaria sphacelata*. Aust. Vet. J. 50:131–132.
4. Hoagland, D. R., and D. I. Arnon. 1950. Water culture method for growing plants without soil. Calif. Agric. Exp. Stn. Circ. 347.
5. Kingsbury, J. M. 1964. Poisonous principles. p. 42–43 *in* J. M. Kingsbury, Poisonous Plants of the United States and Canada, Prentice-Hall, Inc., Englewood Cliffs, NJ.
6. Martinovich, D., and B. Smith. 1972. Kikuyu poisoning in sheep. N.Z. Vet. J. 29:169.
7. Martinovich, D., and B. Smith. 1973. Kikuyu poisoning in cattle. 1. Clinical and pathological findings. N.Z. Vet. J. 21:55–63.
8. Smith, B., and D. Martinovich. 1973. Kikuyu poisoning in cattle. 2. Epizootiological aspects. N.Z. Vet. J. 21:85–89.
9. Walthall, J. C., and R. A. McKenzie. 1976. Osteodystrophia fibrosa in horses at pasture in Queensland: field and laboratory observations. Aust. Vet. J. 52:11–16.
10. Williams, M. C. 1960. Effect of sodium and potassium salts on growth and oxalate content of halogeton. Plant Physiol. 35:500–505.