Reprinted from AGRONOMY JOURNAL
Vol. 71, July-August 1979, p. [illegible]-630

Accumulation of NO_3 and NH_4 in Reed Canarygrass[1]

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ABSTRACT

Recent investigations have shown that reed canarygrass (*Phalaris arundinacea* L.) contains chemical compounds implicated with low palatability, reduced gains, and deaths in cattle. The purpose of this study was to investigate the effects of environmental variables on the accumulation of NO_3 in reed canarygrass. An understanding of the response of this species to its environment will aid in the management and culture of reed canarygrass for production of livestock. Reed canarygrass clones were grown in sand-filled pots in a growth room under the following controlled conditions: temperature, 15 and 30 C; irradiance, 4.2 and 42.0 W/m^2; soil moisture, low medium, and saturated; fertilizer-N, 0, 110, 220, and 440 kg/ha. The clones were cut from meadow sod where the soil, tentatively classified as Silvies series, was Fluventic and Cumulic Haplaquoll—fine-silty, mixed, mesic family. Herbage yields and accumulations of NH_4-N and NO_3-N at the end of three growing periods were evaluated. Increasing temperature from 15 to 30 C increased the rate of growth for plants receiving 42.0 W/m^2. Yields were highest when plants were grown in saturated soil at 30 C with 42.0 W/m^2 and 440 kg N/ha. Concentrations of NH_4 and NO_3 were highest in plants grown in unsaturated soil at 30 C with 4.2 W/m^2 and 440 kg N/ha. When reed canarygrass was grown under these conditions, the NO_3-N concentration increased to 2.43%, 10 times the level (0.21%) considered potentially toxic to livestock.

Additional index words: **(*Phalaris arundinacea* L.), Temperature, Irradiance, Soil moisture, Yield, Nitrate toxicity.**

REED canarygrass (*Phalaris arundinacea* L.) has long been used as a forage and hay crop, especially on swamps or flood plains of sandy, mucky, or peaty nature. Some cattle producers do not use this plant because of its low palatability, low feeding quality as hay, and accumulation of some objectionable chemical compounds.

Recent investigations have shown high correlations between total alkaloid concentrations and palatability (Marten et al., 1973; Kendall and Sherwood, 1975). Hovin and Marten (1975) observed considerable differences in the distribution of specific alkaloids in reed canarygrass cultivars, and Gander et al. (1976) found that clones with N, N-dimethyltryptamine or 2-methyl-1, 2, 3, 4-tetrahydro-B-carboline did not contain their respective methoxy or hydroxy derivitives. They concluded that inverse biosynthetic relationships existed between gramine and the tryptamine and B-carboline alkaloids.

Donker et al. (1976) observed that lambs fed fresh

[1] Cooperative investigation of the AR-SEA-USDA and the Agric. Exp. Stn., Oregon State Univ. Technical Paper No. 4830 of the latter. Received 5 Sept. 1978.

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reed canarygrass did not gain as much per unit of dry matter as those fed reed canarygrass hay. They also determined that freshly cut reed canarygrass contained 69% more alkaloids than the hay. Nevertheless, lambs gained 97 and 48 g/day, respectively, on freshly cut alfalfa and reed canarygrass compared to 63 g/day from alfalfa hay and a loss of 7 g/day from reed canarygrass hay.

Nitrogen fertilizer increased the digestibility of reed canarygrass cut at a grazing stage (Cizek, 1974), but Parmar and Brink (1976) found that tryptamine levels in reed canarygrass were associated with ammonium fertilizers. They further noted that the tryptamine levels in reed canarygrass were closely associated with the incidence of bovine pulmonary emphysema. The tryptamines accumulated in the young plant tissue with chlorophyll but was not found in appreciable quantities in the root, stem, or older leaves.

This paper reports the effects of limited environmental conditions on potentially dangerous accumulations of nitrate in reed canarygrass.

MATERIALS AND METHODS

Reed canarygrass clones were grown in pots under growth room conditions where temperature, irradiance, and soil moisture were limited. Plant plugs (6 cm diam × 6 cm long) cut from meadow sod were refrigerated until placed in pots to commence growth. The soil of the natural meadow, tentatively classified as Silvies series, was Fluventic and Cumulic Haplaquoll—fine-silty, mixed, mesic family. One plug was placed in each pot (15 × 15 × 15 cm) and filled with 2.5 kg of coarse sand. Temperature was controlled by heat from lights, auxillary heat source, fans, and ventilation. Irradiance was controlled with banks of flourescent and incandescent lights programmed for 16 hours of light/day. Soil moisture levels were maintained by adding water to approximate the desired moisture regime. Water was applied by pouring 100 cc of water every 2nd day into pots of the medium-moist regime and 100 cc of water every 4th to 6th day in pots of the low moisture regime. Water was added to water-tight containers as necessary to maintain the saturated regime.

The following levels of control were imposed:

1. Air temperatures of 15 ± 3 and 30 ± 9 C. Fluctuations were associated with light and dark periods.
2. Irradiances of 4.2 and 42.0 W/m^2. The lower light treatment was shaded from direct lighting.
3. Soil moisture of low (approximately 10 bars), medium (approximately 0.3 bar), and saturated (0 bar). Moisture tensions were approximated from pressure chamber values and water content of samples selected from moisture treatments. The exact moisture tensions experienced by the plants were not known because of the method of applying water and because the soil of the intact plug was finer textured than was the potting sand.
4. Ammonium sulfate fertilizer at 0, 180, 360, and 720 mg N/pot. These rates determined for surface area, were equivalent to 0, 110, 220, and 440 kg N/ha.

Treatments were stratified for temperature by time and for irradiance by location and shading. Moisture treatments were stratified within irradiance treatments. Species were randomly located within moisture treatments and fertilizer treatments were randomly allotted within species in three replicated blocks. Plants were harvested at three periods of growth.

The plant materials were dried at 65 C for 48 hours, weighed for yield, and ground for chemical analyses. The micro-Kjeldahl apparatus and techniques of Bremner and Edwards (1965) and Bremner and Keeney (1966) were used, as modified by the Soil Testing Laboratory of Oregon State University, to determine concentrations of NH_4^+ and NO_3^-.

RESULTS AND DISCUSSION

Three days after placement in the growth room, new growth was observed on plants exposed to 42.0 W/m^2, but no new growth was observed on plants receiving 4.2 W/m^2 until the 4th day. Plant growth responses to the environmental conditions were described previously (Gomm, 1978).

Yield. Herbage yields reflect the combined effects of environmental factors on growth characteristics. Statistical analyses indicated the highly significant influence of irradiation and stage of development. They also indicated the significant interactive influence of temperature and radiation. Increasing irradiance from 4.2 to 42.0 W/m^2 significantly increased herbage yields ($P<0.001$), and generally, herbage production of plants grown with 42.0 W/m^2 increased as plants matured (Table 1). Although phenological development was similar for plants grown 85 days at 15 C as compared with those grown 45 days at 30 C, herbage yields of plants grown for 85 days at the lower temperature were much greater. Because of the slow or poor growth of plants at 15 C and 4.2 W/m^2, yield differences due to fertilizer rates and soil moisture under these low temperature-low irradiance conditions were not significant. Responses of plants to fertilizer and soil moisture grown at 30 C and 42.0 W/m^2, however, were highly significant.

Table 1. Herbage yield of reed canarygrass as affected by various growing conditions.

Soil moisture level and fertilizer rate	Dry matter yield at different numbers of growing days (g/pot)					
	15	30	45	45	65	85
kg N/ha	— 30 C and 42.0 W/m^2 —			— 15 C and 4.2 W/m^2 —		
Low						
0	0.40 a*	0.15 a	0.01 a	0.16 a	0.15 a	0.11 a
110	0.23 a	0.18 a	0.40 b	0.16 a	0.21 a	0.19 a
220	0.16 a	0.18 a	0.70 c	0.24 a	0.12 a	0.14 a
440	0.34 a	0.09 a	0.69 c	0.18 a	0.12 a	0.12 a
Avg.	0.28	0.15	0.45	0.18	0.15	0.14
Medium						
0	0.20 a	0.38 a	0.79 a	0.15 a	0.11 a	0.23 a
110	0.20 a	0.21 a	1.34 b	0.32 a	0.14 a	0.11 a
220	0.25 a	0.59 b	1.20 b	0.23 a	0.23 a	0.10 a
440	0.24 a	0.59 b	1.14 b	0.16 a	0.34 a	0.10 a
Avg.	0.22	0.44	0.85	0.21	0.20	0.13
Saturated						
0	0.23 a	0.98 ab	1.23 a	0.23 a	0.07 a	0.07 a
110	0.76 b	1.09 ab	1.22 a	0.40 a	0.03 a	0.08 a
220	0.40 a	1.18 b	2.30 b	0.12 a	0.23 a	0.08 a
440	0.30 a	0.85 a	3.40 c	0.21 a	0.18 a	0.04 a
Avg.	0.35	1.02	2.04	0.24	0.13	0.06
Medium						
0	0.17 a	0.03 a	0.22 a	1.27 b	1.39 b	1.94 a
110	0.18 a	0.04 a	0.34 a	1.11 ab	0.83 a	1.83 a
220	0.25 a	0.07 a	0.19 a	0.66 a	1.42 b	2.34 b
440	0.22 a	0.06 a	0.19 a	0.88 a	1.60 b	2.20 b
Avg.	0.20	0.05	0.23	0.98	1.31	1.66

* Values in the same growth period and moisture level followed by the same letter do not differ at a 0.05 probability level according to Duncan's multiple range test.

Ammonium Concentrations. Regardless of the form in which it is absorbed, N is converted within the plant to the —N, —NH, or $—NH_2$ forms (Tisdale and Nelson, 1966). The reduced N was present in minute amounts because it is quickly synthesized into more complex compounds and ultimately into protein.

Although NH_4-N concentrations in plant tissues were relatively small, differences due to light and temperature treatments were often highly significant and were highest in plants grown with 4.2 $W/^2$ and at 30 C (Table 2). When plants were grown at 30 C with

Table 2. Ammonium-N concentrations in reed canarygrass herbage as affected by various growing conditions.

Soil moisture level and fertilizer rate	NH_4-N in herbage at different nos. of growing days					
	15	30	45	45	65	85
kg N/ha	%					
	30 C and 42.0 W/m^2			15 C and 4.2 W/m^2		
Low						
0	0.02 a*	0.03 a	0.01 a	0.17 a	0.18 a	0.17 a
110	0.03 a	0.03 a	0.02 a	0.33 c	0.38 b	0.43 b
220	0.03 a	0.02 a	0.02 a	0.26 b	0.34 b	0.60 c
440	0.03 a	0.01 a	0.07 b	0.25 b	0.37 b	0.75 d
Avg.	0.03	0.02	0.03	0.25	0.32	0.49
Medium						
0	0.03 a	0.02 a	0.02 a	0.15 a	0.24 ab	0.20 a
110	0.03 a	0.01 a	0.04 a	0.20 a	0.32 b	0.30 ab
220	0.03 a	0.02 a	0.03 a	0.33 b	0.32 b	0.36 b
440	0.02 a	0.03 a	0.05 a	0.42 b	0.16 a	0.38 b
Avg.	0.03	0.02	0.04	0.28	0.30	0.31
Saturated						
0	0.01 a	0.01 a	0.01 a	0.23 a	0.29 b	0.01 a
110	0.01 a	0.01 a	0.01 a	0.26 a	0.09 a	0.24 b
220	0.02 a	0.01 a	0.01 a	0.29 a	0.22 ab	0.13 ab
440	0.02 a	0.02 a	0.02 a	0.28 a	0.19 ab	0.01 a
Avg.	0.02	0.01	0.01	0.26	0.20	0.10
Medium						
0	0.12 a	0.19 a	0.05 a	0.17 a	0.06 a	0.09 a
110	0.20 ab	0.40 b	0.08 a	0.19 a	0.15 b	0.06 a
220	0.17 a	0.62 c	0.22 b	0.24 b	0.11 ab	0.15 b
440	0.29 b	0.65 c	0.17 b	0.23 b	0.15 b	0.16 b
Avg.	0.20	0.46	0.13	0.21	0.12	0.12

* Values in the same growth period and moisture level followed by the same letter do not differ at a 0.05 probability level according to Duncan's multiple range test.

Table 3—Nitrate-N concentrations in reed canarygrass herbage as affected by various growing conditions.

Soil moisture level and fertilizer rate	NO_3-N in herbage at different nos. of growing days					
	15	30	45	45	65	85
kg N/ha	%					
	30 C and 42.0 W/m^2			15 C and 4.2 W/m^2		
Low						
0	0.91 a*	0.68 a	0.01 a	0.66 a	0.68 a	1.21 a
110	0.80 a	0.64 a	0.57 b	0.98 b	0.98 b	1.12 a
220	0.76 a	0.73 a	0.87 c	0.98 b	1.03 b	1.60 b
440	1.06 b	0.75 a	0.94 c	1.10 c	1.22 c	1.08 a
Avg.	0.88	0.70	0.60	0.93	0.98	1.25
Medium						
0	0.56 a	0.70 b	0.42 a	0.81 a	0.84 a	1.04 a
110	0.84 b	0.38 a	0.80 b	0.68 a	1.38 a	1.12 a
220	0.84 b	0.61 b	0.93 bc	1.01 ab	1.16 a	1.43 a
440	1.04 c	1.02 c	0.99 c	1.24 b	0.59 a	1.13 a
Avg.	0.82	0.68	0.78	0.93	0.99	1.18
Saturated						
0	0.12 a	0.01 a	0.01 a	0.22 a	0.17 a	0.23 a
110	0.05 a	0.20 b	0.01 a	0.45 ab	0.17 a	0.01 a
220	0.14 a	0.01 a	0.04 ab	0.37 a	0.60 b	0.33 a
440	0.10 a	0.11 b	0.09 b	0.62 b	0.41 b	0.01 a
Avg.	0.10	0.08	0.04	0.42	0.34	0.14
Medium						
0	1.15 a	1.19 a	1.16 a	0.17 a	0.02 a	0.14 a
110	2.08 b	1.66 b	2.16 b	0.41 b	0.33 b	0.11 a
220	1.87 ab	1.70 b	2.26 b	0.41 b	0.28 b	0.14 a
440	1.50 a	1.76 b	2.43 b	0.55 b	0.25 b	0.22 a
Avg.	1.65	1.58	2.00	0.38	0.22	0.15

* Values in the same growth period and moisture level followed by the same letter do not differ at a 0.05 probability level according to Duncan's multiple range test.

4.2 W/m^2, the NH_4-N level appeared to increase at 30 days then decreased at 45 days.

The NH_4-N concentration of plants grown in saturated soil was generally lower than that of plants grown in unsaturated soils and highest with high rates of N fertilizer (Table 2). At 45 days with 42.0 W/m^2 and 30 C, reed canarygrass fertilized with 440 kg/ha of N contained 0.02, 0.05, and 0.07% NH_4-N, respectively, in the saturated, medium, and low moisture soils.

Nitrate Concentrations. Plants growing in medium-moist soil contained higher levels of NO_3-N when grown at 30 C than at 15 C (Table 3). Also, when grown with 4.2 W/m^2, regardless of temperature, they contained higher concentrations of NO_3-N than they did when grown at 42.0 W/m^2 ($P < 0.001$). Plants grown for 85 days in medium-moist soil at 15 C with 4.2 W/m^2 accumulated NO_3-N concentrations up to 1.43%. This concentration is about seven times greater than the 0.21% level considered dangerous to livestock. With 42.0 W/m^2 at 15 C, however, herbage NO_3-N was within the safe level at 85 days, containing 0.14% NO_3-N with fertilizer-N rates below 220 kg/ha.

The highest NO_3-N concentrations were in plants grown at 30 C with 4.2 W/m^2 and in medium-moist soil (Table 3). Under these high temperature, low irradiance, and moisture conditions, reed canarygrass contained toxic levels of NO_3 at all stages of growth, and the level of toxicity generally increased as fertilized rates increased. At 45 days of growth, the NO_3-N concentration increased from 1.16 to 2.43%, respectively, in herbage samples from unfertilized plants and from those fertilized with N at 440 kg/ha. These concentrations are five to 10 times that considered lethal.

Plants grown in saturated soil with 42.0 W/m^2 and at 30 C contained NO_3-N concentrations significantly lower than those in herbage of plants grown in the drier soils ($P < 0.0001$). Concentrations of NO_3-N generally increased when plants were fertilized, but the increase was not always consistent with increased rates of application (Table 3). Although the NO_3-N concentration appeared to have increased slightly at the high fertilizer rates when plants were grown in saturated soils, differences were small and differences among stages of growth were not significant.

CONCLUSIONS

The accumulation of NO_3 in plants is influenced by the amount of available soil NO_3. Thus heavy application of fertilizer may increase the NO_3 concentration in the resulting dense growth. Since the activation of nitrate reductase is a light-stimulated reaction, it is expected that the NO_3 concentration in lower stems and leaves would be higher than the concentration in leaves exposed to full sunlight. When a stand of reed canarygrass is fertilized, it is probable that the level of NO_3 would be higher in the lowest stems in dense stands than in the lowest stems in stands dominated by relatively short, narrow-leafed plants.

The effect of soil moisture on the concentration of NO_3 was well demonstrated. The normal response is for the NO_3 concentrations to be highest when plants are immature and to decrease as plants mature. However, plants that make their early growth in saturated soils should be low in NO_3 and concentrations should be within the safe range.

The exceedingly high level of NO_3-N accumulated in reed canarygrass when grown under low irradiance and at high temperature indicated the possibility of nitrate poisoning of animals by this species. The probability of nitrate poisoning occurring early in the growing season, however, is low. At that time the soil moisture level is usually high, temperature is relatively low, and shading of the lower stem and leaves is minimal. As the season progresses the probability of NO_3 accumulating in the tissues increases. Growth in low moisture soils, especially after fertilizer application, reduces the amount of radiant energy to reach the lower plant parts, and the high daytime temperatures of late summer produce conditions shown to result in toxic levels of NO_3-N.

Care should be taken in the use of reed canarygrass grown on unsaturated soils. Under drought conditions, or late in the season reed canarygrass plants grown without flood water, especially if heavily fertilized, could be dangerous to livestock.

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