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Herbage Yield and Nitrate Concentration in Meadow Plants as Affected by Environmental Variables

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Abstract

Livestock losses from NO_3 poisoning vary among locations. An understanding of the effect of environmental factors on NO_3 accumulation in meadow plants may aid in management of meadowlands to reduce these losses. Controlled studies were undertaken to determine the effect of soil moisture, temperature, irradiance, and soil fertility on the yield and NO_3 concentrations in herbage tissue of slender sedge (*Carex praegracilis* W. Boott), beardless wildrye (*Elymus triticoides* Buckl.), Nevada bluegrass (*Poa nevadensis* Vasey ex Scribn.) and reed canarygrass (*Phalaris arundinacea* L.). Reed canarygrass consistently yielded higher and contained higher concentrations of NO_3 than the other species. Herbage yields were higher in all plants grown for 45 days at 30°C than in those grown for 45 days at 15°C. Yields were also higher in plants grown with 42.0 W/m^2 than they were in plants grown with 4.2 W/m^2. Concentrations of NO_3 were highest when plants were grown in unsaturated soil, with 4.2 W/m^2, and they increased with fertilization. The NO_3 concentrations obtained could be toxic to cattle (lethal NO_3-N level = 0.21%), especially when the plants were grown at 30°C in dry soils. When meadow plants were grown in saturated soil, the NO_3 concentrations were never high enough to be toxic, even when the plants were heavily fertilized.

The incidence of livestock loss from nitrate poisoning varies among plant species and localities. While some species or types of forage are recognized as potentially dangerous at one location, they may never cause trouble in another location. Areas not previously identified as supporting dangerous types of forage have reported losses after cultural practices were changed or fertilizers were used.

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Nitrogen fertilizer is important for increasing forage yields where grasses are the dominant species. High rates of fertilizer N, however, increase the N content of these plants (Willhite et al. 1955; and Rumburg 1969, 1972). Cattle ingesting such high-N feeds may develop nitrate poisoning. The property of causing nitrate poisoning, however, is not limited to fertilized plants; poisoning can also occur when cattle graze environmentally stressed plants. Primary factors known to affect the accumulation of N in plants include nutrient supply, soil moisture level, temperature, light, soil type, herbicides, and disease (McKee 1962).

Normally, the NO_3 taken from the soil by plants is converted into ammonia, amino acids, protein, and other proteinaceous compounds. The N in herbage often is erroneously considered to be entirely in the proteinaceous forms. Environmental stresses that upset the photosynthesis-respiratory balance can, however, cause dangerous amounts of NO_3 to accumulate in plant tissues (Deinum 1971; and Murata 1969).

Rumburg (1972) observed that the concentration of N in herbage of species native to flood meadows of eastern Oregon increased rapidly after fertilizer was applied. It reached a maximum level in 10-14 days then decreased gradually until the N concentration of the fertilized herbage equaled that of nonfertilized herbage. It is not known how much of the N in the herbage was NO_3-N, how long the NO_3 persisted in the plants, or whether any species accumulated excessive amounts of NO_3. The object of this study was to determine the effects of variations in temperature, irradiance, soil moisture content, and fertilizer rate on yield and NO_3 accumulation in four species important in the flood meadows of eastern Oregon.

Materials and Methods

The study was conducted in a growth room where temperature, irradiance, and soil moisture were controlled in a 4-phase experiment.

Plant plugs (6 cm diameter × 6 cm long) of slender sedge (*Carex praegracilis* W. Boott), Nevada bluegrass (*Poa nevadensis* Vasey ex Scribn.), beardless wildrye (*Elymus triticoides* Buckl.), and reed canarygrass (*Phalaris arundinacea* L.) cut from meadow sod were refrigerated until placed in pots to commence growth. 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, auxiliary heat source, fans, and ventilation. Irradiance was controlled with banks of fluorescent and incandescent lights programmed for 16 hours of light per 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°C±3 and 30°C±9. Fluctuations were associated with light and dark periods.
2. Irradiances of 4.2 and 42.0 W/m². 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 0, 180, 360, 720 mg N/pot. These rates determined for surface area were equivalent to 0, 110, 220, and 440 kg N/ha.

Environmental conditions maintained in the four phases of the experiment were as follows:

Phase 1. Temperature at 15°C; irradiance at 42.0 W/m² and 4.2 W/m²; soil moisture at medium regime.

Phase 2. Temperature at 30°C; irradiance at 42.0 W/m² and 4.2 W/m²; soil moisture at medium regime.

Phase 3. Temperature at 15°C; irradiance at 4.2 W/m²; soil moisture at low, medium, and saturated.

Phase 4. Temperature at 30°C; irradiance at 42.0 W/m²; soil moisture at low, medium, and saturated.

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 NO_3-N analyses. The micro-Kjeldahl apparatus and techniques of Bremner and Edwards (1965) and Bremner and Kenney (1966) were used, as modified by the Soil Testing Laboratory of Oregon State University, to determine concentrations of NO_3-N. All oxidized N was considered to be NO_3-N.

Results and Discussion

Dormancy of the plants was broken by refrigeration, and plants began to grow soon after they were potted and placed in the growth room. All species appeared to grow normally with an irradiance of 42.0 W/m². Bluegrass and wildrye began growth with 4.2 W/m², but many of these plants died within 45 days under this low irradiance and were not included in the NO_3 analysis for phases 1 and 3.

Main Effects

Phase 1—temperature at 15°C, medium moist soil. Canarygrass yielded significantly more than the other species. Bluegrass yielded least, while sedge and wildrye produced intermediate yields (Table 1). The plants grown for 85 days with 42.0 W/m² yielded more than they did at shorter growth periods and lower irradiance level. At this low temperature, fertilizer failed to increase yields.

Table 1. Herbage yield and NO_3-N concentrations in meadow plants grown at 15°C in medium-moist soil.

Treatment	Yield (g/pot)	$(NO_3$-N(%)[1]
Species		
Slender sedge	0.52b[2]	0.35a
Nevada bluegrass	.30a	—
Beardless wildrye	.58b	—
Reed canarygrass	.82c	.64b
Duration of growth (days)		
45	0.37a	0.51b
65	.51b	.39a
85	.78c	.58c
Irradiance level (W/m¹)		
4.2	0.12a	0.78b
42.0	.99b	.22a
Fertilizer rate of N (kg/ha)		
0	0.51a	0.36a
110	.57a	.49b
220	.56a	.56bc
440	.58a	.57c

[1] Analyses of variance NO_3-N included only sedge and canarygrass because so many bluegrass and wildrye plants died, thereby reducing the numbers of data available for these spaces.

[2] Within column and treatment groups followed by different letters are significantly different at $P<0.05$ according to Duncan's multiple range test.

Canarygrass contained higher concentrations of NO_3-N than did sedge. The concentration was higher at 85 days of growth with 4.2 W/m² than it was at shorter growth periods, and it increased with increased rates of fertilizer.

Phase 2—temperature 30°C, medium moist soil. Canarygrass yielded more than the other species, and all species yielded more when grown with an irradiance of 42.0 W/m² than they did when grown with 4.2 W/m² (Table 2). Yield increases with fertilizer rate were small, and only the yield difference between fertilized and unfertilized treatments was statistically significant.

Table 2. Herbage yield and NO_3-N concentration in meadow plants grown at 30°C in medium-moist soil.

Treatment	Yield (g/pot)	NO_3(%)
Species		
Slender sedge	0.15a[1]	0.61a
Nevada bluegrass	.25a	.66a
Beardless wildrye	.21a	.67a
Reed canarygrass	.38b	1.25b
Duration of growth (days)		
15	0.20b	0.88b
30	.15a	.68a
45	.40c	.84b
Irradiance level (W/m³)	0.14a	1.02b
4.2 42.0	.35b	.58a
Fertilizer rate of N (kg/ha)		
0	0.21a	0.45a
110	.24b	.76b
220	.26b	.93c
440	.27b	1.06d

[1] Within column and treatment groups followed by different letters are significantly different at $P<0.05$ according to Duncan's multiple range test.

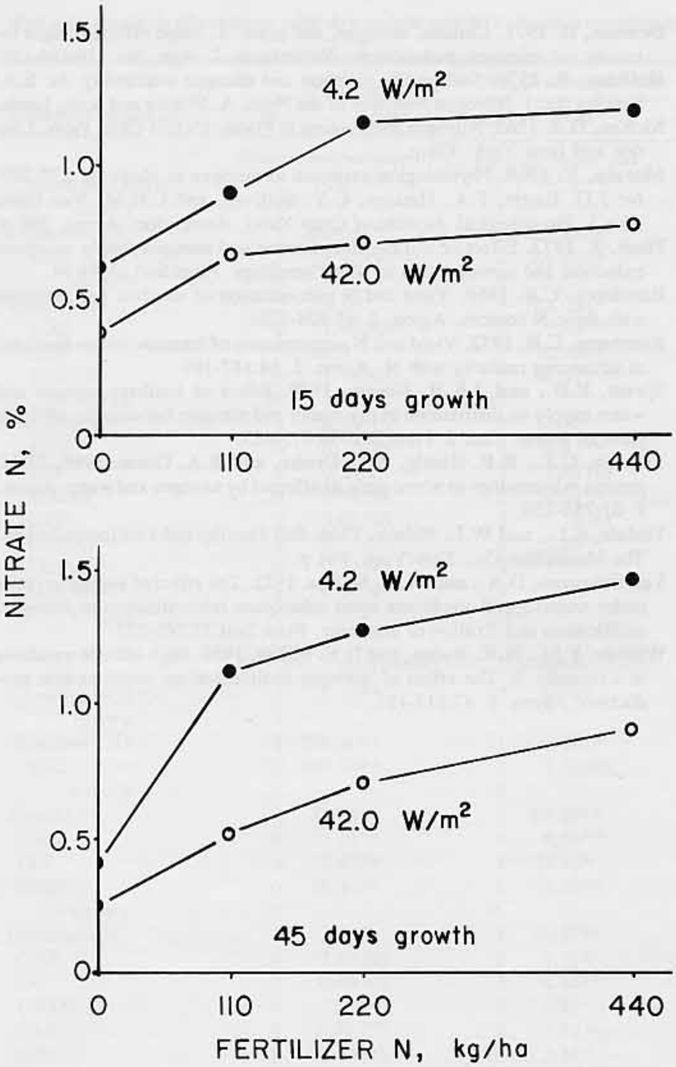

Fig. 1. *Average NO_3-N concentrations in meadow plants grown at 30°C in medium moist soil as affected by irradiance, fertilizer rate, and length of growth period.*

The concentration of NO_3-N in canarygrass was twice as large as the concentration in the other species. The NO_3-N concentration was also greater in plants grown at 4.2 W/m² than it was in those grown at 42.0 W/m² and increased with each increase in fertilizer rate (Fig. 1).

Phase 3—temperature 15°C, irradiance 4.2 W/m². Yields were extremely low from this phase of the experiment. The plants yielded the most at 45 days of growth, and yields decreased at the longer periods of growth (Table 3). Under this low temperature-low light condition, the yield response to fertilizer and soil moisture levels was nonsignificant.

Concentrations of NO_3-N were higher in plants grown for 85 days than for shorter periods and were highest in canarygrass. Plants grown in saturated soil were significantly lower in NO_3-N concentration than those grown in unsaturated soil. Fertilization significantly increased the NO_3-N concentration, but increases were not consistent with increasing fertilizer rates.

Phase 4—temperature 30°C, irradiance 42.0 W/m². All plants appeared to grow well under these high temperature and light conditions. Canarygrass yielded more than the other species, bluegrass yielded more than wildrye, and sedge yielded the least (Table 4). Yields generally increased as plants aged, but the differences in yield at 15 and 30 days of growth were not significant. Yields also increased with increasing rates of fertilizer and soil moisture. It should be noted, however, that the increase with increased soil moisture was due to the high yield of canarygrass in saturated soil. The other species produced more when grown in unsaturated soil than they did in saturated soil (Table 5).

Table 3. Herbage yield and NO_3-N concentrations in meadow plants grown at 15°C with an irradiance of 4.2 W/m².

Treatment	Yield (g/pot)	NO_3-N (%)[1]
Species		
Slender sedge	0.16c[2]	0.34a
Nevada bluegrass	.05a	—
Beardless wildrye	.07b	—
Reed canarygrass	.16c	.80b
Duration of growth (days)		
45	0.14c	0.55b
65	.01b	.51b
85	.08a	.66c
Moisture level[3]		
Low	0.11a	0.74b
Medium moist	.11a	.78b
Saturated	.10a	.20a
Fertilizer rate of N (kg/ha)		
0	0.10a	0.45a
110	.11a	.53b
220	.12a	.68c
440	.11a	.63c

[1] Analyses of variance of NO_3-N included sedge and canarygrass only because so many bluegrass and wildrye plants died, thereby reducing the number of data available for these species.
[2] Within column and treatment groups followed by different letters are significantly different at $P<0.05$ according to Duncan's multiple range test.
[3] Low, about 10 bars; medium moist, about 0.33 bar; and saturated, about 0 bar.

Canarygrass contained the highest concentration of NO_3-N; concentrations in the other species were similar to each other and changed very little through the growing period. Plants grown in saturated soil contained low concentrations of NO_3-N, only 10% as much as that in plants grown in unsaturated soil (Fig. 2).

Interaction Effects

Highly significant differences were detected for growth period, irradiance, moisture, and fertilizer effects on NO_3 concentrations at both temperature regimes. Fertilizer, however did not cause increases in dry weight when plants were grown at 15°C (Table 6). This is an important consideration in that increasing rates of fertilizer N in cold spring weather could increase the concentration of NO_3 in the forage without in-

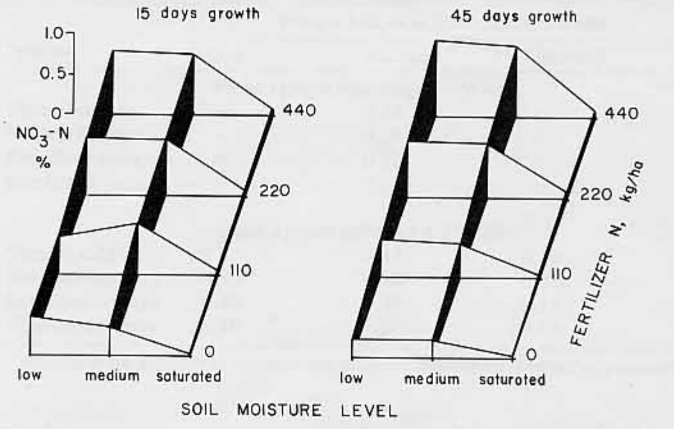

Fig. 2. *Average NO_3-N concentrations in meadow plants grown for 15 and 45 days as affected by soil moisture and fertilizer levels. Plants were grown at 30°C and 42.0 W/m².*

Table 4. Herbage yield and NO_3-N concentrations in meadow plants grown at 30 °C with an irradiance of 42.0 W/m^2.

Treatment	Yield (g/pot)	NO_3-N (%)
Species		
Slender sedge	0.18a[1]	0.37a
Nevada bluegrass	.30c	.35a
Beardless wildrye	.26b	.35a
Reed canarygrass	.69d	.52b
Duration of growth (days)		
15	0.24a	0.43b
30	.27a	.35a
45	.55b	.41b
Moisture level[2]		
Low	0.21a	0.55b
Medium moist	.35b	.58b
Saturated	.49c	.06a
Fertilizer rate of N (kg/ha)		
0	0.29a	0.23a
110	.34b	.33b
220	.36b	.46c
440	.42c	.56d

[1] Within column and treatment groups followed by different letters are significantly different at $P<0.05$ according to Duncan's multiple range test.
[2] Low, about 10 bars; medium moist, about 0.33 bar; and saturated, about 0 bar.

creasing forage yield.

Significant 2-, 3-, and 4-way interactions (Table 6) make determinations of optimum conditions for plant growth and accumulation of NO_3 difficult. These highly significant interactions indicate that the four species grew and accumulated NO_3 differently in response to the different levels of growth period, soil moisture, irradiance, and fertilizer. The problem becomes one of determining which set of conditions will give the highest yield of dry matter and which conditions cause the lowest and highest NO_3 concentrations.

Yield

The species × irradiance × growth period interaction in phase 2 (30°C) was highly significant but in phase 1 (15°C) the interaction of these factors was nonsignificant. This indicates that the different species responded differently to irradiance levels at the two temperature regimes. With irradiance of 42.0 W/m^2, plants grown in phase 4 at 30°C for 15 days developed at a faster rate and were phenologically similar to those grown in phase 1 at 15°C for 45 days. Maximum yield at 45 days of growth was also higher for plants grown at 30°C than for those grown at 15°C.

Table 5. Yield of meadow plants grown for 45 days at 30°C with 440 kg N/ha.

Species	Dry weight of herbage at 3 moisture levels (g/pot) Low	Medium	Saturated
	Phase 4: plants received 42.0 W/m^2		
Slender sedge	0.43c[1]	0.33b	0.21a
Nevada bluegrass	0.30b	0.59c	0.18a
Beardless wildrye	0.40a	0.54b	0.39a
Reed canarygrass	0.69a	1.14b	3.40c
	Phase 2: plants received 4.2 W/m^2		
Slender sedge	—	0.16	
Nevada bluegrass	—	0.35	
Beardless wildrye	—	0.08	
Reed canarygrass	—	0.19	

[1] Means within species followed by different letters are significantly different at $P<0.05$ according to Duncan's multiple range test.

The highly significant 2-, 3-, and 4-way interactions of phase 4 (Table 6) and yield response (Table 4) indicate that reed canarygrass grown for 45 days at 30°C with 42.0 W/m^2 would yield more herbage when the soil was saturated and when fertilizer was applied at the 440 kg N/ha rate than it would at lesser moisture and fertilizer levels. The 3-way interaction of growth period × moisture × fertilizer indicates also that the plants as a group may have responded similarly to the reed canarygrass but probably not to the same degree. Dry weight measurements, however, show that yields of sedge declined as the soil moisture level increased, and bluegrass and wildrye yielded more from the meduim moisture treatment than from the saturated (Table 5).

NO_3-N Accumulation

The dominating effect of soil moisture on the concentration of NO_3 was well demonstrated (Fig. 2); the usual response is for the concentration of NO_3 to be high in plants fertilized with high rates of N. Plants grown in saturated soil, however, were low in NO_3 and within the safe range—less than 0.21% NO_3-N, (Binns 1956)—even when fertilized with 440 kg N/ha.

High NO_3-N concentrations in the plants from phase 2 (Table 2) indicate that plants may accumulate higher concentrations of NO_3 when grown at 30°C than when grown at 15°C as in phase 1 (Table 1). Species × irradiance and species × moisture interaction (Tables 6, 7 and 8) also indicate that plants grown at 4.2 W/m^2 in less than saturated soil gave the highest concentrations of NO_3. Although it is not possible to determine the significance level of the species × temperature × irradiance × moisture × fertilizer interaction from these experiments, the results indicate that of the species tested maximum concentrations of NO_3 will occur in reed canarygrass when heavily fertilized and grown at high temperature, low irradiance, and unsaturated soil conditions. Meadows will be lowest in NO_3 when composed of sedge, bluegrass, and wildrye species and when temperature is relatively low, irradiance high, and the soil is saturated.

Several authors have reported studies that help explain the results of this experiment. D'Aoust and Tayler (1969), and Tisdale and Nelson (1966) have reported on the importance of adequate soil moisture, temperature, and oxygen for microbial action in the soil and for the efficient use of the N by plants; and Van Schreven and Sieben (1972) showed that the NO_3 content of the soil decreased and NH_4 increased after the soil was waterlogged. Because of the reducing and denitrifying conditions prevailing in saturated soil and because the fertilizer applied was $(NH_4)_2SO_4$, the NH_4 probably was not oxidized to NO_3 before absorption. Apparently, with an irradiance of 42.0 W/m^2 the NH_4-N absorbed by the plants was readily assimilated into protein compounds.

The accumulation of NO_3 in plants is influenced by the amount of available soil NO_3, and the shading caused by dense growth as that resulting from heavy applications of fertilizer may cause NO_3 concentrations to increase further. The characteristic growth habit of a plant could, therefore, affect its accumulation of NO_3. Since the activation of nitrate reductase is a light-stimulated reaction, the NO_3 concentration is expected to be higher in plant parts that are grown in shade than in plant tissues exposed to full sunlight. Fertilization of a dense stand of reed canarygrass is therefore more likely to result in a high concentration of NO_3 in the lower stems than is fertilization of

Table 6. Analysis of variance with dry weight and NO_3-N concentration as dependent variables.

Source of variation	Dry weight df	Dry weight *F* ratio	NO_3-N concentration df	NO_3-N concentration *F* ratio
	Phase[1]: temperature 15°C, medium moist soil			
Growth period (G)	2	19.31**	2	15.20**
Error a	4		4	
Irradiance (I)	1	280.63**	1	848.88**
GXI	2	28.50**	2	61.02**
Error b	6		6	
Species (S)	3	24.04**	1	164.23**
GXS	6	1.46 NS	2	11.03**
IXS	3	14.92**	1	91.35**
GXIXS	6	1.16 NS	2	4.92*
Error c	36		12	
Fertilizer (F)	3	0.56 NS	3	12.10**
GXF	6	0.75 NS	6	2.87**
IXF	3	0.23NS	3	1.45 NS
GXIXF	6	0.48 NS	6	4.09**
SXF	9	0.65 NS	3	2.28 NS
GXSXF	18	0.93 NS	6	5.45**
IXSXF	9	0.71 NS	3	3.51*
GXIXSXF	18	0.84 NS	6	2.28*
Error d	114		72	
	Phase 2: temperature 30°C, medium moist soil			
Growth period (G)	2	75.95**	2	8.74*
Error a	4		4	
Irradiance (I)	1	779.80**	1	130.38**
GXI	2	140.74**	2	1.52 NS
Error b	6		6	
Species (S)	3	38.65**	3	67.29**
GXS	6	13.61**	6	6.56**
IXS	3	27.82**	3	24.33*
GXIXS	6	16.34**	6	5.20**
Error c	36		36	
Fertilizer (F)	3	2.93*	3	60.57**
GXF	6	2.02 NS	6	4.73**
IXF	3	0.84 NS	3	4.84**
GXIXF	6	3.07**	6	2.52*
SXF	9	1.48 NS	9	1.82 NS
GXSXF	18	1.44 NS	18	2.54*
IXSXF	9	1.19 NS	9	1.46 NS
GXIXSXF	18	1.33 NS	18	1.81*
Error d	144		144	

Source of variation	Dry weight df	Dry weight *F* ratio	NO_3-N concentration df	NO_3-N concentration *F* ratio
	Phase 3: temperature 15°C, irradiance 4.2 W/m²			
Growth period (G)	2	11.28*	2	68.48**
Error a	4		4	
Moisture (M)	2	1.51 NS	2	272.64**
GXM	4	4.59*	4	19.47**
Error b	12		12	
Species (S)	3	95.64**	1	474.20**
GXS	6	6.11**	2	4.09*
MXS	6	2.70*	2	38.29**
GXMXS	12	2.22*	4	7.83**
Error c	54		18	
Fertilizer (F)	3	1.02NS	3	14.54**
GXF	6	3.09**	6	3.59**
MXF	6	0.33 NS	6	0.57 NS
GXMXF	12	1.56 NS	12	4.44**
SXF	9	0.94 NS	3	2.24 NS
GXSXF	18	1.98**	6	5.56**
MXSXF	18	0.64 NS	6	2.13 NS
GXMXSXF	6	2.12**	12	2.88*
Error d	216		108	
	Phase 4: temperature 30°C, irradiance 42.0 W/m²			
Growth period (G)	2	228.37**	2	55.79**
Error a	4		4	
Moisture (M)	2	326.22*	2	1578.86**
GXM	4	86.29**	4	15.31**
Error b	12		12	
Species (S)	3	407.65**	3	67.20**
GXS	6	130.57**	6	13.67**
MXS	6	120.74**	6	17.54**
GXMXS	12	39.46**	12	4.65**
Error c	54		54	
Fertilizer (F)	3	26.61**	3	112.76**
GXF	6	19.89**	6	8.17**
MXF	6	5.11**	6	21.97**
GXMXF	12	5.84**	12	4.26**
SXF	9	16.04**	9	1.80 NS
GXSXF	18	16.19**	18	3.47**
MXSXF	18	8.61**	18	2.58**
GXMXSXF	36	16.08**	36	3.47**
Error d	216		216	

*,** Significant at the 0.05 and 0.01 level of probability, respectively.

Table 7. Nitrate concentration in meadow plants when grown for 45 days at 30°C with 440 kg N/ha.

Species	Percent NO_3-N at 3 soil moisture levels: Low	Medium	Saturated
	Phase 4 plants receiving 42.0 W/m²		
Slender sedge	1.32b	1.07b	0.01a
Nevada bluegrass	0.83b	0.61b	0.08a
Beardless wildrye	0.77b	0.96b	0.04a
Reed canarygrass	0.94b	0.99b	0.04a
	Phase 2 plants receiving 4.2 W/m²		
Slender sedge	–	0.82	–
Nevada bluegrass	–	0.84	–
Beardless wildrye	–	1.80	–
Reed canarygrass	–	2.43	–

[1] Means within species followed by different letters are significantly different at $P<0.05$ according to Duncan's multiple range test.

Table 8. Nitrate concentration in meadow plants when grown for 45 days at 15°C with 440 kg N/ha[1].

Species	Percent NO_3-N at 3 soil moisture levels: Low	Medium	Saturated
	Phase 1 plants receiving 42.0 W/m²		
Slender sedge	–	0.27	–
Nevada bluegrass	–	0.39	–
Beardless wildrye	–	0.32	–
Reed canarygrass	–	0.55	–
	Phase 3 plants receiving 4.2 W/m²		
Slender sedge	0.25	0.47	0.14
Nevada bluegrass	0.70	0.54	0.23
Beardless wildrye	0.40	0.70	0.11
Reed canarygrass	1.10	1.24	0.62

[1] Because of the mortality of bluegrass and wildrye plants only mean values are presented.

stands dominated by sedge or bluegrass, which are relatively short, narrow-leafed plants.

Conclusion

Since NO_3-N concentrations as low as 0.21% can be lethal to cattle, meadow plants grown with an irradiance of 4.2 W/m^2 or grown in dry soil may contain toxic concentrations of NO_3, especially if fertilized. Even plants grown with an irradiance of 42.0 W/m^2 and fertilized with N at rates of 110 kg/ha or higher should be considered potentially dangerous for 6 weeks or longer after the fertilizer is applied. When meadow plants are grown in saturated soil, however, the plant tissue apparently does not accumulate toxic concentrations of NO_3.

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