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Authors: Skinner, Geoffrey, Emerman, Steven H., and Leichty, Reid J.

Source: BIOS, 79(2) : 61-66

Published By: Beta Beta Beta Biological Society

URL: [https://doi.org/10.1893/0005-3155\(2008\)79\[61:FUBCAU\]2.0.CO;2](https://doi.org/10.1893/0005-3155(2008)79[61:FUBCAU]2.0.CO;2)

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# Fluorine uptake by cultivated and uncultivated grasses: implications for co-evolution between grasses and grazers

Geoffrey Skinner, Steven H. Emerman and Reid J. Leichty

*Department of Biology and Environmental Science, Simpson College, Indianola, Iowa 50125*

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**Abstract.** Fluorine has no known physiological role in grasses, yet is an essential nutrient for grazing animals. The evolution of an uptake mechanism for F must be examined in the context of the coevolution of grasses and grazers. The objective of this study was to determine whether uncultivated grasses buffer the uptake of F in a range acceptable for grazing animals and whether cultivated grasses have lost this buffering mechanism. The uncultivated grasses big bluestem (*Andropogon gerardii* Vitman), little bluestem (*Schizachyrium scoparium* (Michx.) Nash) and switchgrass (*Panicum virgatum* L.), and the cultivated grasses wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.) and oats (*Avena sativa* L.), were grown in a greenhouse and watered with NaF at F concentrations in the range 0–9 mg/kg. Total F concentrations of plant shoots were determined by the alkali-fusion method. Average F contents for uncultivated grasses (29 mg/kg for big bluestem, 26 mg/kg for little bluestem, 25 mg/kg for switchgrass) were below the upper limit for dairy cattle (40 mg/kg), while average F contents for cultivated grasses (51 mg/kg for wheat, 97 mg/kg for rye, 99 mg/kg for oats) were well above the upper limit for cattle. For the uncultivated grasses, plant F content did not correlate with F concentration of irrigation water ( $R^2 = 0.13$  for big bluestem,  $R^2 = 0.14$  for little bluestem,  $R^2 = 0.22$  for switchgrass), while correlations were moderate to good for cultivated grasses ( $R^2 = 0.61$  for wheat,  $R^2 = 0.52$  for rye,  $R^2 = 0.52$  for oats).

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## Introduction

Fluorine has no known physiological role in grasses, yet is an essential nutrient for the bones and teeth of grazing animals (Weinstein, 1977; Taiz and Ziegler, 1998). The question as to why grasses have evolved an uptake mechanism for F must be examined in the context of the coevolution of grasses and grazers.

Adaptive responses of grasses that enable them to evade, resist or tolerate herbivory include the presence of silica in epidermal cells, below-ground perennating organs, and aboveground production in excess of that which decomposes in a single year (Stebbins, 1981; Anderson, 1982, 1990, 2006; Coughenour, 1985). It has also been suggested that moderate grazing increases the productivity of grasses (McNaughton, 1979, 1993; Owen, 1981). Grazers increase the availability of inorganic nutrients by converting recalcitrant forms of nitrogen to urea, which is easily mineralized to plant-available forms (Knapp et al., 1999).

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Correspondence to: Steven H. Emerman, 1023 64<sup>th</sup> Street, Des Moines, Iowa, 50311, phone: (515) 277–6813; e-mail: SHEmerman@crosspaths.net

Grazers remove older, less productive leaf tissue and thereby increase light and moisture for younger, more productive leaf tissue. Of course, excessive grazing decreases the productivity of grasses (Frank et al., 1998). The result is a complex interaction between grasses and grazers in which grasses have evolved in a way that both discourages and encourages herbivory. This type of interaction occurs throughout ecology. The existence of thorny plants with brightly colored fruit is an excellent example.

The objective of this study was to investigate the possibility that grasses evolved an uptake mechanism for F as a means of encouraging grazing. The following questions were asked:

1. Do uncultivated grasses buffer the uptake of F, so that the total F concentration of plant shoots is relatively independent of the F concentration of irrigation water?
2. Has the buffering mechanism been lost in cultivated grasses so that the total F concentration of plant shoots is a function of the F concentration of irrigation water?

Positive answers to the above questions would be difficult to explain in the absence of a coevolution between grasses and grazers. The questions were addressed by growing in a greenhouse the cultivated grasses wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.) and oats (*Avena sativa* L.), and the uncultivated grasses big bluestem (*Andropogon gerardii* Vitman), little bluestem (*Schizachyrium scoparium* (Michx.) Nash) and switchgrass (*Panicum virgatum* L.). The grasses were irrigated with NaF in the range 0–9 mg F/kg water, which represents the range of F concentrations typically occurring in ground-water. (The USEPA maximum contaminant level (MCL) for fluoride in drinking water is 4 mg/kg with a suggested maximum contaminant level (SMCL) of 2 mg/kg (Fetter, 1994)).

There have been no published reports of field or greenhouse experiments comparing uptake of F by cultivated and uncultivated grasses. Braen and Weinstein (1985) reported on greenhouse experiments on uptake of F by orchard grass (*Dactylis glomerata* L.) and red maple seedlings (*Acer rubrum* L.) growing in F-contaminated soil. These authors reported greater foliar F in

orchard grass than red maple at high soil F concentration and, in general, there was low correlation between foliar F and either soluble soil F or labile soil F. The vast majority of research on grasses and F involves the ability of grasses to absorb atmospheric F pollutants (Bunce, 1985; Kessabi and Assimi, 1984; Craggs and Davison, 1985; Notcutt and Davies, 1989, 2001).

## Materials and Methods

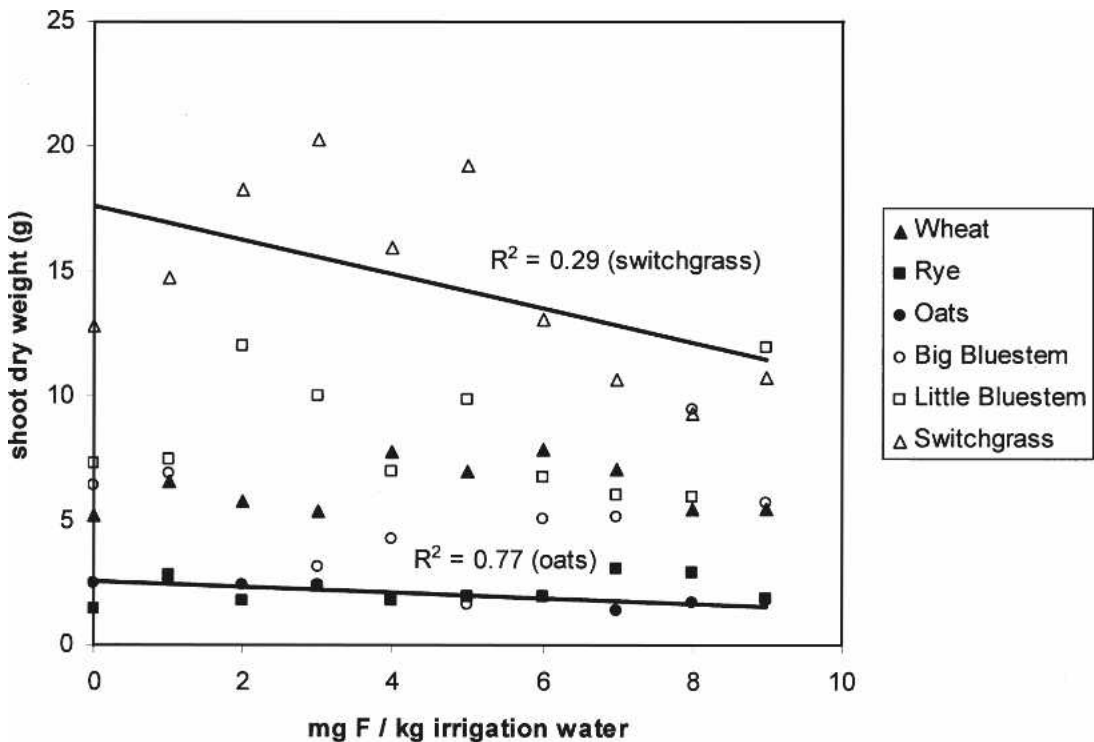
In the spring of 2006 three seeds of wheat, rye and oats were sown in 6-inch (15.24-cm) pots in a greenhouse. The potting medium was 40% loam from an organic garden, 40% peat and 20% perlite (all percentages by volume). Weed seeds in the loam were killed by baking at 82°C for 20 minutes. The greenhouse temperature was maintained at 21–32°C and supplementary lighting was provided from 5 am–7 pm. NaF treatments began two weeks after sowing. The healthiest plant in each pot was retained, while the other two were removed. Each species was separated into 10 treatment groups with five pots per treatment. Plants were watered with NaF in concentrations of 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9 mg F/kg deionized water. Plants received no other source of water after the initiation of treatments. All plants were well-watered and water was applied until it drained freely from the pots. Plant shoots were harvested eight weeks after treatments. Plant shoots were dried at 70 °C for 72 hours and shoot dry weights were recorded to within  $\pm 0.001$  g. All five plants of a given species and treatment were combined for measurement of total F. In the fall of 2006 live plants of big bluestem, little bluestem and switchgrass were purchased from Prairie Nursery (Westfield, Wisconsin, USA) and grown in the same pots with fresh potting medium. All aspects of growing uncultivated grasses were the same as growing cultivated grasses, except that treatments began one week after potting and no plants were removed from pots. The greenhouse was sufficiently warm and well-lit to prevent any prairie grasses from going into dormancy. There were no visible symptoms of F toxicity (Weinstein, 1977) in any of the cultivated or uncultivated grasses.

Total F of dried shoots was determined using the alkali-fusion method (McQuaker and Gurney, 1977; Frankenberger et al., 1996). Dried shoots of a given species and treatment were homogenized in a coffee grinder and passed through a 300- $\mu\text{m}$  sieve. After homogenization 0.5 g of plant sample was combined with 6 mL of 17 M NaOH in a Ni crucible and placed in an oven at 150°C. The crucible was removed after solidification of the NaOH, which required one hour. The crucible was then transferred to a muffle furnace set at 300°C. The temperature was gradually raised to 600°C and the sample was fused for 30 minutes. The crucible was removed from the furnace, allowed to cool, and 10 mL of deionized water was added. The crucible was heated slightly to aid in dissolution of the NaOH fusion cake. Concentrated HCl was added to reduce the pH to 8 to 9, which required 8 mL of HCl. The sample was allowed to cool again and vacuum filtered through Whatman No. 40 filter paper. The F concentration of the filtrate was

then measured using the Hach DR/890 spectrophotometer. Fluorine concentration of filtrate  $C_F$  (mg/L) was converted to F concentration of plant shoot  $C_P$  (mg/kg) using the formula

$$C_P = C_F \frac{1.8 \times 10^{-3} \text{ L}}{5 \times 10^{-4} \text{ kg}}$$

(The volume of liquid added to the dried soil was 10 mL water + 8 mL HCl = 18 mL. All water associated with the 6 mL of NaOH was driven out during oven-heating.) Two blanks were used throughout the entire procedure and the results were identical within the precision of the spectrophotometer. The blank F concentration, equivalent to 16 mg F/kg dry plant matter, was subtracted from all measurements of total plant F. A sample of Kentucky bluegrass (*Poa pratensis* L.) was collected from the college lawn and homogenized. Two replicates were analyzed from the sample yielding 68 mg F/kg dry plant matter. The results of the two replicates were



**Figure 1.** Dependence of shoot dry weight of plant on fluorine concentration of irrigation water for cultivated (closed symbol) and uncultivated (open symbol) grasses. Shoot dry weights are uncorrelated with fluorine concentration of irrigation water for wheat ( $R^2 = 0.02$ ), rye ( $R^2 = 0.09$ ), big bluestem ( $R^2 = 0.05$ ) and little bluestem ( $R^2 = 0.002$ ).

again identical within the precision of the spectrophotometer.

Soil pH was recorded separately for all 75 pots of uncultivated grasses. After harvesting the shoots, the soil from each pot was frozen and then thawed, homogenized and air-dried prior to measurement. Soil pH was measured by combining 20 g of air-dried soil with 40 mL of 0.01 M CaCl<sub>2</sub> and stirring for 30 minutes. The mixture was allowed to sit after stirring, but the soil was so fine-grained that no clear supernatant appeared even after 60 minutes. Therefore, the soil pH was measured by placing the pH electrode at a uniform depth within the mixture immediately after stirring.

In all cases, statistical significance was established by the Student's unpaired t-test at  $P < 0.05$ .

Results

The toxic effects of F on grasses were highly variable. Oats showed the clearest F toxicity with a linear correlation ( $R^2 = 0.77$ ) between shoot

dry weight and F concentration of irrigation water (Figure 1). There was not a significant correlation between shoot dry weight and F concentration of irrigation water ( $R^2 = 0.29$ ) for switchgrass. For all other grasses, there were no toxic effects of F ( $R^2 = 0.02$  for wheat,  $R^2 = 0.09$  for rye,  $R^2 = 0.05$  for big bluestem, and  $R^2 = 0.002$  for little bluestem). Shoot weights tended to be higher for the uncultivated grasses, which was expected since the cultivated grasses were started from seed (Table 1). However, the difference between the shoot dry weights of wheat and big bluestem was not statistically significant ( $P = 0.12$ ).

Fluorine uptake was much higher among the cultivated grasses than among the uncultivated grasses (Table 1). The differences in total F concentration among the uncultivated grasses were not statistically significant (big bluestem vs. little bluestem:  $P = 0.51$ , big bluestem vs. switchgrass:  $P = 0.43$ , little bluestem vs. switchgrass:  $P = 0.86$ ). The total F concentration in rye and oats was over three times greater than the total F concentration in the uncultivated grasses. For the

Table 1. Average dry shoot weights and total F concentrations for cultivated and uncultivated grasses.

Grass	Shoot Dry Weight (g)	Total F Concentration
		(mg F / kg dry plant matter)
<u>Cultivated Grasses</u>		
Wheat	a 6.3 ± 0.3	a 51 ± 6
Rye	b 2.2 ± 0.2	b 97 ± 3
Oats	b 2.0 ± 0.1	b 99 ± 4
<u>Uncultivated Grasses</u>		
Big Bluestem	a 5.0 ± 0.8	c 29 ± 4
Little Bluestem	c 8.4 ± 0.8	c 26 ± 2
Switchgrass	d 15 ± 1	c 25 ± 2

All values are average ± standard error. Differences between values in the same column with the same letter are not statistically significant at the 95% confidence level, according to the Student's unpaired t-test.

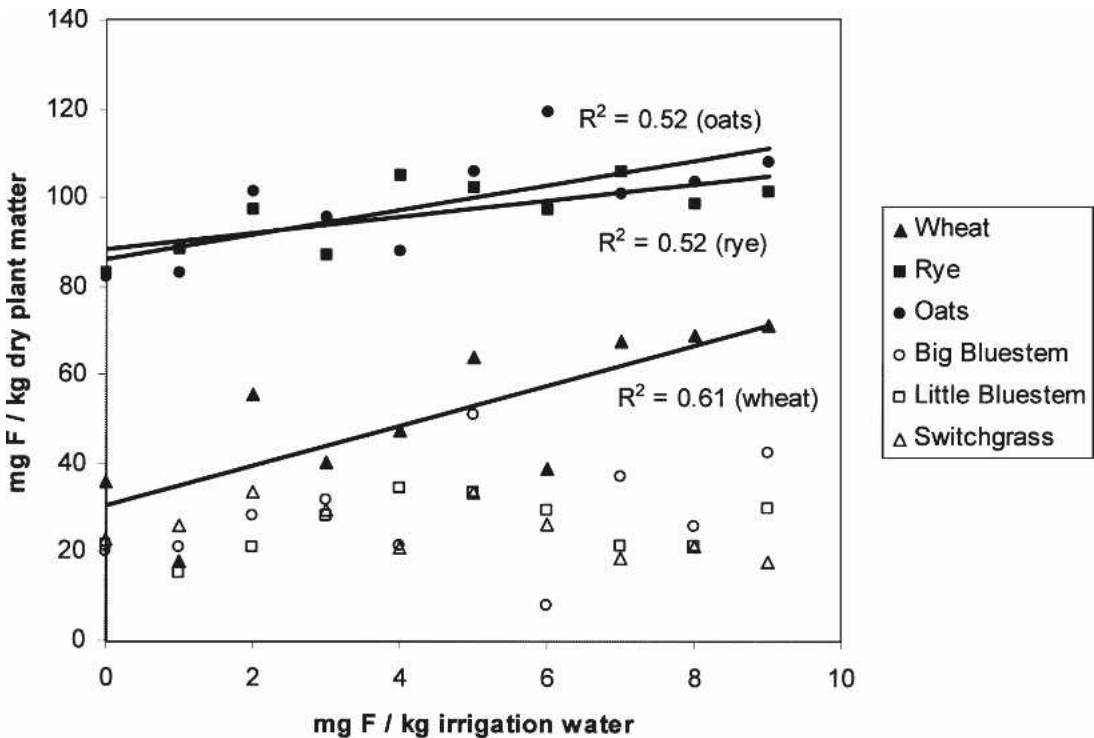
cultivated grasses, the linear correlations between total F concentration of plant shoots and F concentration of irrigation water were moderate to good and statistically significant ( $R^2 = 0.61$  for wheat,  $R^2 = 0.52$  for rye,  $R^2 = 0.52$  for oats) (Figure 2). However, for the uncultivated grasses, total F concentration of plant shoots and F concentration of irrigation water were not significantly correlated ( $R^2 = 0.13$  for big bluestem,  $R^2 = 0.14$  for little bluestem,  $R^2 = 0.22$  for switchgrass).

The average soil pH for uncultivated grasses and F concentrations of irrigation water was  $5.14 \pm 0.02$  (S.D. = 0.19). All pots had acidic soil with the soil pH ranging from 4.55 to 6.23. The variations in soil pH among uncultivated grasses were not statistically significant (big bluestem vs. little bluestem:  $P = 0.08$ , big bluestem vs. switchgrass:  $P = 0.83$ , little bluestem vs. switchgrass:  $P = 0.13$ ) and there were not significant linear correlations between soil pH and F concentration of irrigation water (big bluestem:  $R^2 = 4 \times 10^{-6}$ , little bluestem:  $R^2 = 0.0002$ , switchgrass:  $R^2 =$

0.002) or between soil pH and F concentration of plant shoots (big bluestem:  $R^2 = 0.04$ , little bluestem:  $R^2 = 0.02$ , switchgrass:  $R^2 = 0.01$ ).

## Discussion

Previous greenhouse experiments on a variety of cultivated plants have shown that F uptake is not a function of the total F content of soil, but depends upon the soil type, Ca and P contents of soil, and soil pH (Prince et al., 1949; Hurd-Karrer, 1950; Brewer, 1965). However, as is the case with many other elements, plant uptake does not normally depend upon the total elemental concentration in soil, but the concentration of an element in bioavailable forms. There are procedures for measuring water-soluble F,  $\text{CaCl}_2$ -extractable F (Frankenberger et al., 1996) and resin-extractable F (Braen and Weinstein, 1985), but it has not been demonstrated that these procedures measure bioavailable F, or that these measurements of F correlate with F contents of



**Figure 2.** Comparison of dependence of fluorine concentration of plant on fluorine concentration of irrigation water for cultivated (closed symbol) and uncultivated (open symbol) grasses. Fluorine concentrations of plants and irrigation water are uncorrelated for uncultivated grasses ( $R^2 = 0.13$  for big bluestem,  $R^2 = 0.14$  for little bluestem,  $R^2 = 0.22$  for switchgrass).

plants. In this study, soil characteristics and soil pH were uniform, and F was added in a highly bioavailable form. Therefore, it became clear that uncultivated grasses buffered the uptake of F, while the F uptake of cultivated grasses was a linear function of added bioavailable F.

The upper limit for F concentration of grass fed to dairy cattle is 40 mg/kg (Bunce, 1985). The lower limit and optimum ranges have not been determined. The F concentrations of the uncultivated grasses fall within the acceptable range for cattle, even for high levels of bioavailable F added to the soil. The implication is that selective grazing by herbivores has caused grasses to evolve in such a way as to provide adequate, but not excessive levels of F to grazing animals. On the other hand, grasses exposed to airborne F can have F concentrations as high as 700 mg/kg (Kessabi and Assimi, 1984). High levels of airborne F have not been a major part of the evolutionary history of grasses and grasses have not evolved a mechanism to prevent the accumulation of F through leaves. By contrast, F concentrations of cultivated grasses were well above the upper limit for cattle (Table 1). Average F contents for rye and oats were nearly 2.5 times the upper limit. The F contents for all three cultivated grasses exceeded the upper limit for cattle even when there was no added F and plants were simply exposed to the F present in the potting medium (Figure 2). The implication is that cultivating these grasses for other characteristics has eliminated their ability to buffer the uptake of F.

**Acknowledgements.** This research was partially supported through an undergraduate research grant from the Beta Beta Beta Research Scholarship Foundation.

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Received 7 September 2007; accepted 20 December 2007.