Exhibit 1c, # 15

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Effect of SO₄-S Fertilization on Se Concentration of

Alfalfa (Medicago sativa L.)¹

D. T. Westermann and C. W. Robbins²

ABSTRACT

Selenium is not required for plant growth, but is necessary for the prevention of white muscle disease and other low-Se related animal disorders. Areas in the Pacific Northwest that produce forages low in Se are nearly identical to those known to be S deficient. While SO.S has been shown to inhibit SeO.'Se uptake by plants, the effect of S fertilization on Se uptake by plants, the effect of S fertilization on Se uptake by plants growing on low-Se soils where other forms of Se may exist has not been evaluated. This was examined on eight alfalfa (Medicago sativa L.) experimental sites where S fertiliza-tion was a variable in suthern I dobe. Viside ware more tion was a variable in southern Idaho. Yields were measured, and plant samples were taken from the first harvest of alfalfa (0.1 bloom) and analyzed for total Se and S, and Se uptake.

Suffur fertilization significantly reduced forage Se con-centrations at four of the eight experimental sites. For-ages at seven sites contained less than 0.1 ppm Se before S fertilization, which was further reduced by the S fer-tilization. The decrement in Section which was tilization. The decrease in Se concentration mainly re-flected a dilution effect caused by a growth response to the S fertilization. No direct relationship was apparent between forage Se and S levels. This study showed that S fertilization of S deficient, low-Se soils to increase forage production may increase the incidence of Se deficiency in animals. Thus ranchers, cattlemen, and agronomists should become aware of this potential problem and pro-vide protective measures against Se deficiency in their livestock.

Additional index words: S deficiency, Animal nutrition, Se uptake.

•ONSIDERABLE evidence has accumulated in the C last decade that dietary Se is required to prevent white muscle disease in livestock (2, 10, 18). Minimal dietary Se concentrations range from 0.03 to 0.10 ppm Se, depending upon vitamin E level, sulfulamino acids, and antioxidants. Some investigators have suggested 0.10 ppm Se in dry forages as a minimal safe level for preventing white muscle disease and other low Se-related disorders, whereas dietary Se levels above 3 to 5 ppm are toxic to livestock.

Factors influencing Se uptake by plants are: 1) plant species, 2) chemical forms of Se present, 3) soil physical and chemical factors, and 4) interaction with other ions, particularly S. Early work by Hurd-Karrer (11, 12) showed that S compounds decreased Se uptake by promoting increased S uptake. Later studies showed that SO_4 -S strongly inhibited SeO_4 -Se absorption and partially inhibited SeO_8 -Se absorption (15, 20). These findings have been used in numerous attempts to reduce Se toxicity to plants and animals on seleniferous soils (16, 23).

An additional aspect of the S-Se antagonism is the possible effect of S fertilization on Se uptake by plants growing on soils low in available Se. Gissel-Nielsen (9) showed that plant uptake of applied Se

was highest where a fertilizer low in S was used. In the Pacific Northwest, the areas of S deficiency (4) are nearly the same as those that produce forages containing less than 0.10 ppm Se (6, 8). To evaluate the effect of S fertilization on the Se concentration of forages, we collected alfalfa (Medicago sativa L.) samples from a S fertilization study in progress and analyzed them for Se (22).

METHODS AND MATERIALS

The experimental sites were located in mountain valleys in the Idaho counties of Camas, Custer, and Teton. These valleys are at elevations of 1520 to 1850 m, have 60 to 90 frost-free days, and receive 180 to 380 mm of annual precipitation, mostly as snow. Soils are acid to slightly alkaline and formed from a variety of parent materials. The soil series, suborder classifica-tion, and some physical and chemical characteristics of the 0 to tion, and some physical and chemical characteristics or the 0 to 30-cm soil layers for each of the experimental sites are shown in Table 1. Soil pH was determined with a glass electrode on a saturated soil paste and organic matter was determined by the method of Walkley and Black (21). Soil SO_4 -S was extracted by 0.1 *M* LiCl and determined by the methylene-blue reduction method (13). Plant available soil Se was not determined; how ever, these areas have been identified as producing forages low in Se (8). Experiment 6, 7, and 8 were sprinkler irrigated; the remainder were not, although moisture was not limiting for the first harvest.

Sulfur was applied as gypsum (Table 2) the fall before sam-pling on the nonirrigated sites and in the spring of the sam-pling year on the irrigated sites. Forage samples were obtained and yields were measured from the first harvest at 0.1 bloom.

Table 1. Selected soil physical and chemical characteristics of the 0 to 30-cm soil layer for each experimental site.

Site		Suborder	Organic			
No.	Series	classification	pН	matter	SO4 - S	
				5.	ppm	
1	Riceton 1	Typic Argixeroll	5.6	2.01	2,46	
2	Simenton 1	Typic Argizeroll	5, 8	1, 52	0, 50	
з	Simonton gl	Typic Argizeroll	5.6	1, 38	1.59	
4	Riceton 1	Typic Argixerol1	5.5	1, 69	2, 06	
5	Riceton 1	Typic Argixarol1	5.6	1, 80	0.85	
6	Gini gl	Typic Haplargid	7, 4	1.42	2, 34	
7	Bereniceton sil	Xeric Torriorthent	7.6	2, 98	6.62	
8	Tetonia all	Pachic Cryoboroli	6.4	2.38	1, 54	

Table 2.	Effect	of SO ₃ -S	fertilization	on dry s	matter	yields,	Se
concent	rations	and upt	ake, and S c	oncentratio	on of a	lfalfa.	

Experimental	Fertilizer S0 ₄ -S	Forage yteld	Forage composition		
site No.			Se	·	5
	kg/ha	mi/ha	ppm	ng/ha	io.
1	0	2,46 a*	0, 079 a"	194 a.	0, 112 a'
	34	3,47 a	0, 015 b	62 b	0, 195 b
2	0	0.31 a	0,080 a	25 a	0,065 a
	22	2.02 b	0,055 a	111 b	0,184 b
3	0	I. 1А а	0,051 a	58 a	0,070 a
	45	4, 21 b	0,026 b	109 b	0,156 b
ł	0	3,90 a	0,022 a	86 a	0, 142 a
	22	4,31 a	0.025 a	109 n	0, 216 b
5	0	і,кія	0, 032 a	58 a	0,065 a
	22	3.к3 b	0, 023 b	88 ab	0,116 b
	67	3.3-й с	0, 019 b	104 b	0,175 e
ŕ	0	4,21 a	0, 163 a	686 a	0, 186 a
	34	4,61 a	0, 108 b	198 a	0, 229 a
7	0	ના,∔ાર	0,087 a	388 a	0, 221 a
	34	નુ,મરક્ષ	0,114 a	555 a	0, 229 a
8	1)	2,240 a	0, 024 a	54-4	0, 048 a
	22	31,63 b	0, 011 a	4-2 a	0, 234 b
	67	31,90 b	0, 020 a	76 a	0, 264 b

Means followed by same letter within an experiment are not significantly different at the 0, 05 level.

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Kimberly, ID 83941.

The plant samples were oven-dried at 55 C, ground, and an-alyzed for Se (1) and S (19). All plant data are given on an oven-dried basis and are the averages of four replications. Other essential nutrient elements were maintained at adequate levels.

RESULTS AND DISCUSSION

The Se concentrations in the alfalfa ranged from very low to adequate levels for animal nutrition (Table 2). Using 0.10 ppm as the minimal concentra-tion of Se in forages required for normal animal nutrition, forages from seven of the eight experimen-tal sites were low enough to cause possible Se deficiencies in animals, and of these, forages from three sites contained less than 0.05 ppm Se before S fertilization. Only the alfalfa from site 6 had an adequate level of Se for animal nutrition using this criteria. Sulfur fertilization significantly reduced the Se concentration in four experiments. This reduction was independent of the initial Se concentration. There was a nonsignificant increase in Se concentration from S fertilization on site 7. An earlier study showed that SO₄-S increased Se concentration in alfalfa on a low Se alkaline soil well-supplied with SO4-S (7). A similar mechanism may be operative here.

Sulfur fertilization tended to increase total Se uptake (mg/ha) on all sites except 1 and 6 (Table 2). Factors responsible for this difference may include a greater proportion of SeO₄-Se forms and a lack of plant-available forms of Se in soil horizons below the zone of SO4-S enrichment on sites 1 and 6. Most of the decrease in the Se concentration resulted from a dilution effect since yields were generally increased by S fertilization. However, the dilution effect appears to have been modified by increased root growth in areas of plant-available Se and by SO4-S inhibition of Se absorption.

No relationship was shown between plant S and Se levels. However, there may be a relationship on a given site, but no conclusions can be drawn from our limited data. Also, there were no apparent relationships between plant Se levels and soil textures or organic matter levels. Plant Se levels were higher at the higher soil pH's, particularly at sites 6 and 7. Selenate forms of Se are favored by higher pH's and are absorbed by plants in greater amounts than selenite forms (5), which may partially account for the greater amounts of Se in the forages on these two sites. In addition, the level of plant-available Se may be naturally higher in these soils or may have been altered by past P fertilization practices (17).

Our data help explain earlier observations that the incidence of white muscle disease was higher in areas where gypsum was being applied to increase forage yields (10, 18). Also of possible concern is the relative level of S compared to that of Se in forages. Studies have shown that high dietary S levels may interfere with the biological availability of Se (3). While most of the reported studies were not with indigenous S, the increased level of plant S resulting from S fertilization may aggravate the problem of low Se levels in animal nutrition.

The data presented in this paper indicate that S fertilization of low-Se soils sometimes reduces the Se levels in forages. Extensive areas in the northeastern and southeastern Coastal States have been shown to produce forages generally low in Se (14) and many of

these areas are known to require S fertilization for maximum yields (4). The summer grazing areas at the higher elevations in he Pacific Northwest are also low in Se (8). Sulfur ferilization to increase forage yields on these areas or on areas of winter feed production may increase the incidence of Se deficiency in livestock. Livestock managers should therefore be aware of possible Se deficiencies in young livestock and provide for protective measures.

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