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Effect of Phosphorus Fertilization on the Selenium Concentration in Alfalfa (*Medicago sativa*)[1]

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ABSTRACT

A study was conducted to determine the effect of P fertilization on the Se concentration in alfalfa. Adding P to the soil increased the Se concentration in alfalfa grown in the greenhouse on six of 14 soils from the northwestern United States. The Se concentration increase in alfalfa resulting from P addition was noted on some alkaline and some acid soils. Phosphorus addition increased the availability to alfalfa of both native and applied Se in the Portneuf silt loam. Applying 160 kg P/ha either as H_3PO_4 or concentrated superphosphate to Gooding sandy loam in the field increased the Se concentration in alfalfa from a level marginal for animal requirements to an adequate level.

Additional Index Words: **white muscle disease (WMD), P response, P concentration in alfalfa, P × Se interaction.**

THE DISCOVERY that Se is required to prevent white muscle disease (WMD) in calves and lambs (15) stimulated Se research activity. One area of research has been concerned with the minimal Se concentration required in the diet to prevent WMD (1, 9, 10, 11, 18). Results from studies in this research area indicate that the critical deficiency level in the diet is actually a range from 0.03 to 0.07 ppm, depending upon the vitamin E level and other factors. Some investigators have suggested that 0.10 ppm may be a reasonable practical level. A second area of research has been concerned with determining the Se concentration in forage and hay crops throughout the United States, and has provided maps (5, 7, 13) delineating areas of different Se concentrations in forage and hay. Unfortunately, unexplainable farm-to-farm variability required labelling some areas on these maps as variable in Se concentration.

The available information on the Se concentration in soils (14), the different Se forms and their reactions in soils, and the Se uptake by plants (4, 6, 8, 12, 17) indicated that some farm-to-farm variability in the Se concentration in forage resulted from different management practices. Crop rotations and fertilizer practices used in the variable areas indicated that some of the variability may be related to P fertilization. We investigated the Se concentration in P fertilizer materials and the associated uptake by plants (17), and results established that some P fertilizers contain sufficient Se to enhance concentrations in plants. However, the proportion of the P fertilizers containing significant amounts of Se appeared too small to explain much of the observed variability in the field.

This paper reports the effects of P fertilization on the uptake of indigenous and applied Se, and the resulting Se concentrations in alfalfa (*Medicago sativa* L. Ranger).

MATERIALS AND METHODS

Five separate investigations, four in the greenhouse and one in the field, were conducted. These will be referred to as Studies 1, 2, 3, 4, and 5 for identification purposes. Procedures and materials applicable to all studies will be discussed first, followed by a discussion of treatments and experimental procedures associated with specific studies.

General Procedures and Materials

Portneuf silt loam was used exclusively in Studies 1, 2, and 3, and was one of 14 soils used in Study 5. The soil for Study 1 was collected from one site, and that for Studies 2 and 3 from another, but all measured properties were the same. The available P determined by $NaHCO_3$ extraction (16, 19) was 7.5 ppm on a scale where values above 10 ppm are probably adequate. Soil from still a different site was used in Study 5, and it will be discussed later. The available P levels in the Gooding sandy loam at the field study site (Study 4) were 8.8 and 6.0 ppm for the 0- to 15- and 15- to 30-cm depths, respectively. All soils used in Studies 1, 2, 3, and 4 were P deficient. Both the Portneuf and Gooding soils have pH values from 7.8 to 8.2 and contain free $CaCO_3$, particularly below the 15-cm depth.

Pots used in the four greenhouse studies each contained 3 kg of soil. All pots were placed in a water bath table where the water temperature was maintained at about 21C. Selenium-free water was added according to pot weight to maintain between 20 and 100% of available water.

Alfalfa (*Medicago sativa* L. Ranger) was grown in all studies, and it was the principal crop sampled in mapping studies. Rooted cuttings were transplanted to all greenhouse pots, four per pot in Studies 1 and 5, and three per pot in Studies 2 and 3. Alfalfa crops were harvested at approximately 1/10 bloom, both in the greenhouse and the field. All samples were dried at 50C, ground to pass a 2-mm sieve, and analyzed for Se (2).

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The interval between harvests in the greenhouse was about 30 days. Field plots were sampled 48 days after application of P materials. Selenium-free fertilizer materials were used in Studies 2, 3, 4, and 5. All five studies were set up in triplicate randomized complete block designs.

Specific Treatments and Procedures

Study 1—This study was carried out simultaneously with our investigation of the plant uptake of Se contained in P fertilizer materials (17). Treatments were: (i) control, (ii) raw rock phosphate at a rate sufficient to provide 22 ppm P and 0.008 ppm Se, (iii) raw rock phosphate treated with enough H_3PO_4 to provide 79 ppm P and 0.008 ppm Se, (iv) raw rock phosphate treated with H_2SO_4 applied at a rate sufficient to provide 44 ppm P and 0.016 ppm Se, and (v) raw rock phosphate treated with H_3PO_4 equivalent to the H_2SO_4 used in (iv) to provide 156 ppm P and 0.016 ppm Se.

Study 2—The treatments were: 0, 40, and 80 ppm P as concentrated superphosphate at Se levels of 0, 0.25, and 0.50 ppm applied as H_2SeO_3.

Study 3—Treatments were: (i) control, (ii) no P applied until after the second alfalfa crop was removed and then 80 ppm P applied as concentrated superphosphate by distributing the pellets into five holes about 5 to 7 cm deep, and (iii) 80 ppm P applied initially.

Study 4—Field plots, 4m by 5m, in an established alfalfa stand on Gooding sandy loam were treated with P immediately following removal of the second alfalfa crop in 1969. Treatments were: (i) control, (ii) concentrated superphosphate broadcast on the surface at a P rate of 160 kg/ha, and (iii) H_3PO_4 shanked into the soil approximately 10 cm deep and about 30 cm apart at a P rate of 160 kg/ha.

Study 5—Soils were collected from 14 locations in the Northwest and prepared for a greenhouse study as previously described. The soils and some characteristics are given in Table 3 (Results section). Available P was determined on those soils having pH values of 7.0 and above by $NaHCO_3$ extraction (16, 19). The acid soils were extracted with NaOAc for determining available P (The extraction method is used by the University of Idaho Soil Testing Laboratory and it has been adapted from several published methods). Response to P applications generally occurs on soils with extractable P levels < 10 ppm when using these methods. Treatments were (i) control, and (ii) 100 ppm P applied as concentrated superphosphate, except that 50 ppm P was added later to both treatments on Blodgett and Deschutes, and 100 ppm P was added to both treatments on the Jory (see Results section).

Table 1—The Se concentration in alfalfa grown on Portneuf silt loam at different applied levels of P and Se in raw and treated rock phosphate

Material	P rate	Se rate	Alfalfa crop 1	2	3	4	5
			Se concentration				
	ppm	ppb	ppm*				
Control	0	0	0.043 a	0.051 a	0.050 a	0.037 a	0.036 a
Raw rock phosphate	22	8	0.048 a	0.054 a	0.059 ab	0.040 a	0.038 a
Raw rock phosphate + H_3PO_4	79	8	0.085 bc	0.138 b	0.129 c	0.109 bc	0.102 c
Raw rock phosphate + H_2SO_4	44	16	0.063 ab	0.105 b	0.092 b	0.086 b	0.071 b
Raw rock phosphate + H_3PO_4	156	16	0.101 c	0.130 b	0.147 c	0.130 c	0.115 c

* Numbers within each crop followed by different letters differ significantly at the 5% level by Duncan's multiple range test.

RESULTS

Results from Study 1 indicated that increasing the P applied at a given Se application rate increased the Se concentration in alfalfa (Table 1). Selenium concentrations were significantly higher in all alfalfa crops where 79 ppm P was applied than where only 22 ppm was applied at the Se rate of 0.008 ppm. The same pattern was observed at the 0.016 ppm Se rate when the P level was increased from 44 to 156 ppm. Phosphorus applications also increased yields as expected.

The Se concentration was significantly increased by each P application increment in all four alfalfa crops where no Se was applied in Study 2 (Fig. 1*A*). Phosphorus application also increased the Se concentration in alfalfa where 0.25 and 0.50 ppm Se were applied even though the Se concentrations from these treatments were 10 to 30 times greater than where no Se was applied (Fig. 1*B* and 1*C*). Alfalfa yield and P concentration in the alfalfa were increased by applying P to the soil as expected from the soil tests.

Results from Study 3 showed that applying P immediately following the second harvest of alfalfa increased the Se concentration in subsequent crops (Fig. 2*A* and 2*B*). The P effect was manifested where no Se was added and where 0.50 ppm was applied, although the Se concentration was 10 to 30 times greater where 0.50 ppm Se was applied. The same Se concentration resulted in the third

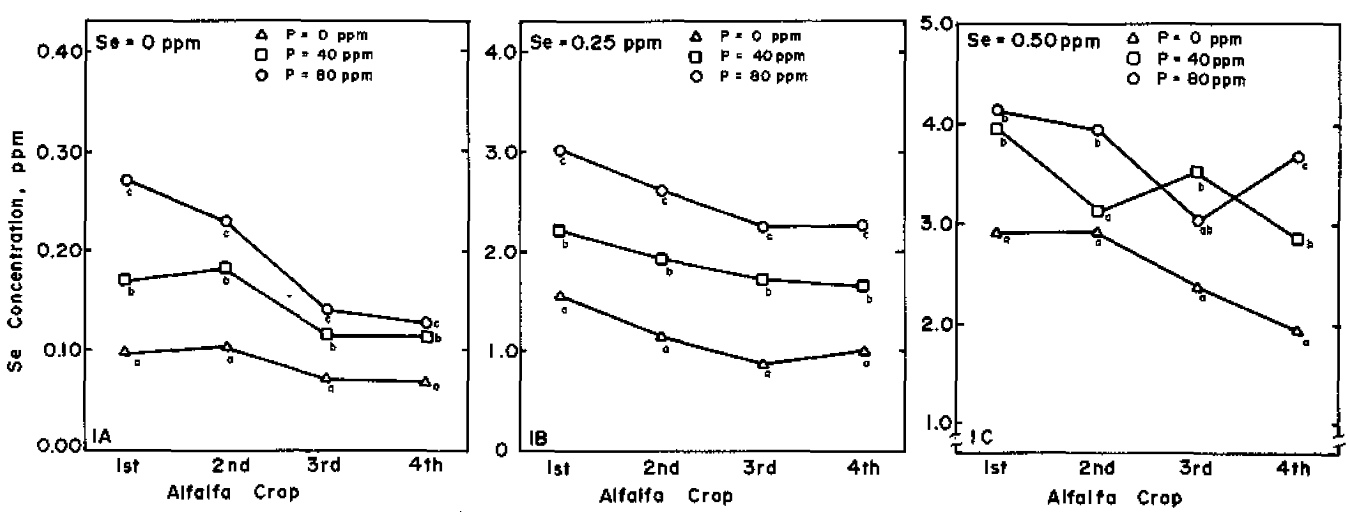

Fig. 1—The Se concentration in alfalfa grown on Portneuf silt loam. Different letters by the data points for each crop indicate significant differences by Duncan's multiple range test at the 5% level.

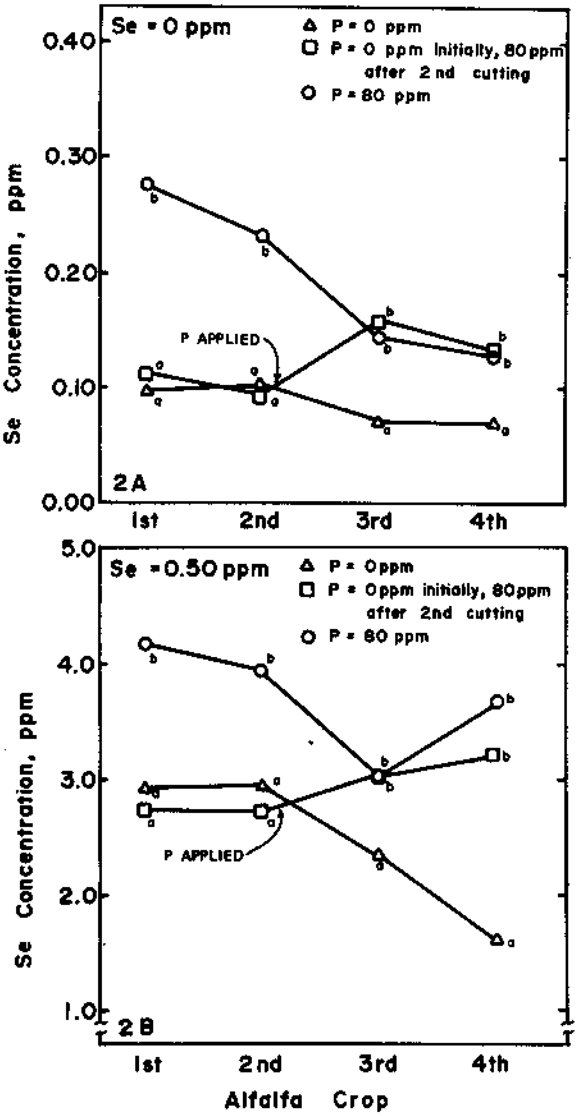

Fig. 2—The Se concentration in alfalfa grown on Portneuf silt loam. Different letters by the data points for each crop indicate significant differences by Duncan's multiple range test at the 5% level.

and fourth alfalfa crops where the P was applied after the second crop as where the P was applied before cropping at both Se levels.

Applying P to an established alfalfa stand in the field on Gooding sandy loam (Study 4) increased the Se concentration in alfalfa from marginal to adequate levels for livestock (Table 2). Injecting H_3PO_4 was more effective than broadcasting concentrated superphosphate. The P concentration in alfalfa was also increased.

Results from Studies 1 through 4 indicated that applying P to P-deficient calcareous soils increased the Se concentration in alfalfa grown on those soils. Study 5 was conducted to determine if the P × Se interaction occurred on other soils. The pH values of the soils used ranged from 5 to 8. Some soils were P-deficient and others were not, according to soil test values. All soils with pH values above 7.0 contained free $CaCO_3$.

Alfalfa seedlings transplanted into the 0 ppm P treatment of the Blodgett, Jory, and Deschutes soils exhibited acute P deficiency symptoms soon after transplanting. In fact, it appeared that the plants would not survive. Therefore 50 ppm P were added to both treatments on the Blodgett and Deschutes soils, changing the treatments to 50 and 150 ppm P. Both treatments on the Jory received 100 ppm, changing the treatments to 100 and 200 ppm. This P was applied by dropping concentrated superphosphate into several holes punched 5 to 7 cm into the soil. The alfalfa responded to the additional P on all three soils. Selenium concentrations in alfalfa were increased by the higher P level over the lower P level on all three soils.

Table 2—Selenium and P concentrations in alfalfa grown on Gooding sandy loam with and without applied P

Source	Applied P	Plant tissue analysis: Se	Plant tissue analysis: P
	kg/ha	ppm	%
Control	0	0.070 a	0.223 a
0-48-0	160	0.107 b	0.247 b
H_3PO_4	160	0.149 c	0.293 c

* Numbers in the same column followed by different letters differ significantly at the 1% significance level by Duncan's multiple range test.

Phosphorus fertilization increased the Se concentration in alfalfa grown on 6 of the 14 soils studied (Table 3). A significant alfalfa yield response was obtained on 8 of the 14 soils. A Se concentration increase and a yield response to added P resulted on five soils. Two of these, the Portneuf and the Scism, were calcareous, and the other three, the Blodgett, Jory, and Deschutes, were acid. In addition to these significant responses, there was a definite trend towards both a yield response and a Se concentration increase on the Purdam, Nyssaton, and Walla Walla.

The Madras-1963 and the Madras-1963-Se soils were included in the study for two purposes. The first was to determine the effects of applying P on the Se concentration in alfalfa, and the second was to determine the effects of a Se treatment made in 1963 (3). The Madras 1963-Se was treated in the field with 1.0 ppm Se as Na_2SeO_3 injected into the soil in water solution in 1963. The Madras-1963 was an adjacent area in the field that received no Se. The data (Table 3) indicate that there are long lasting effects of the 1.0 ppm Se application. The Se concentration in alfalfa from the Se-treated soil was greater after eight cropping seasons than it was from the adjacent untreated soil. Phosphorus applications in the greenhouse did not change the Se concentration in alfalfa grown on these soils.

An attempt was made to determine if applying P increased the extractable Se on soils where the P × Se interaction was observed. Fifty-gram samples of the Portneuf, Minidoka, Blodgett, Jory, Deschutes, and Madras soils, both untreated and with 160 ppm P added, were incubated at a moisture level near field capacity and at room temperature for 48 hours. They were then extracted by shaking with 500 ml of 0.01 *N* $CaCl_2$ for 16 hours and filtered The extracts were concentrated and the Se concentrations were determined (2). We found no differences in the Se concentrations between the untreated and P treated samples. This was true for both soils on which the interaction was evident and where it was not. Two to three times more Se was extracted from the calcareous Portneuf and Minidoka than from the other soils in the study.

Table 3—Mean Se concentrations in alfalfa grown on 14 soils at two levels of applied P, and the significance of the Se concentration differences and yield responses. The collection site, pH, and available P for the soils are also shown

Soil	Site	pH paste	Available P§	Se concentration, Applied P 0	Se concentration, Applied P 100	Significance of increase: Se conc.	Significance of increase: Yield
			ppm	ppm	ppm		
Portneuf sil	Kimberly, Ida.	7.9	9.1	0.057	0.086	*	**
Minidoka sil	Twin Falls, Ida.	7.7	27.2	0.062	0.059	ns	ns
Purdam sil	Nampa, Ida.	7.7	9.0	0.096	0.107	ns	**
Scism sil	Nampa, Ida.	7.9	25.8	0.078	0.093	**	*
Greenleaf sil	Caldwell, Ida.	7.7	21.3	0.190	0.180	ns	ns
Nyssaton sil	Homedale, Ida.	7.7	23.8	0.074	0.084	ns	ns
Madras sl	Madras, Ore.	7.0	18.5	0.090	0.090	ns	*
Blodgett sl†	Woodside, Mont.	5.2	4.8	0.103	0.206	**	**
Jory cl†	Lewisberg, Ore.	5.7	1.1	0.156	0.214	**	**
Palouse sil	Pullman, Wash.	5.4	18.4	0.113	0.110	ns	ns
Walla Walla sil	Pullman, Wash.	5.9	4.6	0.097	0.117	**	ns
Deschutes sl†	Richmond, Ore.	5.7	5.4	0.076	0.098	**	**
Madras sl - 1963‡	Madras, Ore.	5.7	10.9	0.048	0.049	ns	**
Madras sl - 1963-Se‡	Madras, Ore.	5.8	8.6	0.065	0.069	ns	ns

* Significant at 5% level.
** Significant at 1% level.
† P applied to Blodgett and Deschutes was 50 and 150 ppm, and to Jory was 100 and 200 ppm (see "Results" in text).
‡ Refers to previous study discussed in the text.
§ Soils with pH ≥ 7.0 were extracted with $NaHCO_3$. Acid soils were extracted with NaOAc.

DISCUSSION

Interaction between two elements in the soil relative to their uptake by plants has been observed many times. Generally the exact mechanisms for such interactions are not known. The interaction between P and Se has been shown to occur, but like many other interactions, the exact mechanism is not known. Results from this study partly characterize the interaction and establish important implications of it.

One possible explanation for the P × Se interaction might be as follows: Evidently P and Se compete for some reactions in the soils. When Se is involved in these reactions, the products apparently render Se unavailable, or nearly so, to plants. When P is added to the system, it evidently replaces some Se in certain reaction products, forcing the replaced Se to react in such a way that it is more available to plants; hence the Se concentration in these plants is increased. However, we could not detect differences in extractable Se when comparing P treated and untreated soils.

Another possible explanation for the P × Se interaction is that P applications may stimulate plants to absorb more Se because of greatly increased root proliferation. Most of the nutrient absorption is by new root growth. The more prolific the root system, the greater is the root-soil contact or the volume of exploration involving actively absorbing roots. This greater root-soil contact may result in sufficient additional Se uptake to bring about the observed Se concentration increases in plants. Selenium concentration increases and yield responses tended to occur on the same soils, and most of these soils were P-deficient. The relative Se concentration increases observed in the greenhouse when P was added tended to decrease with successive crops, indicating that root proliferation may be a primary factor in the P × Se interaction. The application of P to the field plots caused a greater relative increase in Se in the plants than was noted in most of the greenhouse studies. This also points to increased root proliferation as an important factor.

The P × Se interaction mechanism functions similarly with native and added Se. A 40-ppm increment in applied P increased the Se concentration in first crop alfalfa about 0.08 ppm where no Se was applied, compared to an increase of 0.7 to 1.0 ppm where 0.25 or 0.50 ppm Se was applied.

Gardner (11) has reported an apparent increase in the incidence of Se deficiency in livestock following increased use of phosphate fertilizers in parts of Australia. In our work, P applications did not result in any significant decreases in Se concentration in alfalfa. Therefore, it would appear that any increased incidence of Se deficiency in livestock following increased use of P fertilizers would not be an effect of P, per se.

The differences in Se concentration in crops on different soils in an area mapped as variable in the Northwest (5, 13) are typified by the Se concentrations for 14 soils where no P was applied (Table 3). Differential use of P fertilizers may also account for some of the variability in areas mapped as variable. On some soils, for example—the Gooding, the use of P fertilizers may increase the Se concentration in forage from a low or marginal level for livestock to a level considered adequate for cattle and sheep.

The importance of the P × Se interactions in relation to animal nutrition and the prevention of WMD depends upon the general Se concentration in plants. There are some areas where P fertilizers are applied and yet all forage samples contain Se concentrations below 0.10 ppm (5, 13). In these regions the interacting effects of P on Se may not be of sufficient magnitude to bring about adequate Se concentrations in forage for livestock, but some exceptions should be expected. There are also regions where the Se concentration in all forage samples is above minimal requirements for livestock. In these regions, the P × Se interactions are not important in animal nutrition. The interaction can be most important to the rancher and livestock feed producer in marginal Se areas. Maintaining an adequate P level in the soil will help to insure high crop yields and adequate nutritional concentrations of both P and Se when these crops are used for livestock feed.

In conclusion, results of our investigations have shown that applying P to some soils increases the Se concentration in alfalfa. Thus there is a P × Se interaction that may be important in some areas where WMD losses occur.

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