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Nutrient Interaction Effects on Yield and Chemical Composition of Spinach and Green Beans

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ABSTRACT

Green beans and spinach were grown in field plots in a $2^3 \times 3$ factorially arranged split plot design to study the effect of soil fertility treatments on the chemical composition of the edible plant parts. Two levels each of lime, phosphorus, and potassium and three levels of nitrogen were established on a Mexico silt loam soil that was initially low in fertility.

No green bean yield response from the treatment was obtained because of pre-planting management and weather. Significant yield increases of spinach were obtained from all main treatments; nutrient interaction effects on yield were also observed. NO_3-N, Ca, K, Mn, Zn, Mg, Na, and P concentrations in the edible parts were determined. The concentrations of most of these elements in both vegetables were affected by soil treatment. The net effect of any one soil treatment depended on the levels of the other treatments.

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J. R. BROWN, V. N. LAMBETH, and D. G. BLEVINS[1]

INTRODUCTION

In recent years considerable effort has been expended in studying the influence of fertility practices on plant composition. Objective of this effort has been to develop practices which would lead to the maximum yield of nutritious food. In addition, minimization of toxic products in the edible portions of the plants should be achieved. Recent work at this station has reinforced the age old concept that the balance between elements in the soil has important influences upon plant composition (Brown and Smith 1966, 1967).

The evaluation of the effects of fertility practices is difficult as the method used to measure these effects may influence the results. Fertility level of the soil influences the degree to which the genetic potential of the plant is utilized so yield is related to the genetic potential as well as the fertility. Thus, yield should not be the sole measurement used to evaluate practices *if* knowledge is desired about the effect of fertility variables on the pathway to the measured yield.

Determination of the chemical composition of the plant can provide some insights into the fertility effects. As this type of knowledge accumulates, plant scientists will be better equipped to predict the effect that a given soil fertility level will have on a plant.

The work reported herein was done as a cooperative project between the Departments of Agronomy and Horticulture and the National Canners Association. The work was in part a continuation of a study of the soil fertility level's influence on the level of nitrates in vegetables (Brown and Smith 1966, 1967) and in part a study into the factors affecting shelf life of canned vegetables.

The overall objective of the study was to determine the influence which varying levels of soil Ca, P, K, and N has on the shelf life of canned spinach and green beans. This bulletin reports the effects of the soil treatment on yield and chemical composition of the vegetable at harvest.

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LITERATURE REVIEW

Nitrogen Fertilization and Nitrates

High soil N levels from N fertilization increase the nitrate content of plant tissue (Brown and Smith, 1966, 1967; Hanway and Englehorn, 1958; MacLeod, 1965; Perez and Story, 1960; Smith and Sund, 1965; Wright and Davison, 1964). Brown and Smith (1966, 1967) found that the use of fertilizer N in excess of 50 pounds per acre caused nitrate accumulation in most vegetables.

In their review, Wright and Davison (1964) treated comprehensively the subject of nitrates in relation to plants and animals. The review indicated that K additions increased nitrate accumulation in solution culture experiments but had little effect in field experiments. .

MacLeod (1965) found that for a given N treatment, increased K fertilizer rates tended to decrease the NO_3-N content of several forage species.

Phosphorus fertilization of soils increased the nitrate content of plants in some experiments while in others it decreased the nitrate content, had no effect, or had variable effects.

Molybdenum and Mn deficiencies have caused accumulation of nitrates in plants (Wright and Davison 1964). Molybdenum has shown to be the metallic component of the enzyme nitrate reductase, with Mn performing a similar function in hydroxylamine reductase (Wright and Davison, 1964).

Nitrogen fertilization may increase growth of plant tops and roots, thus having a diluting effect on concentration of other elements or it may increase foraging capacity of the root system and absorption of some elements such as phosphorus (Grunes, 1964; Tserling, 1965).

In pot experiments with spinach, application of N increased the yield of leaves much more than K applications (Ehrendorfer 1964). The K content of grasses was decreased by N fertilization (MacLeod, 1964). Kresge and Younts (1963), working with the N:K ratio of applied fertilizer as related to yield and nutrient content of orchardgrass, found that N:K ratios narrower than 2.4:1 were necessary for maximum yields.

Effect of Liming on Composition

Linin *et al.* (1964) found that liming had no appreciable effect on pod yields of beans on non-saline soils at either low or high fertility levels. Reith (1965) reported that liming acid soils in humid, temperate climates usually increased the percentage of Ca, Mg, and P in crops but this increase did not occur if liming produced substantial increases in yield. Liming often has a marked effect on micronutrient uptake by plants (Beeson *et al.,* 1948; Tisdale and Nelson, 1966). Lime treatments of the soil significantly decreased the levels of Cu, Co, Mn, Fe, and Zn and increased Mo content of peanut tissue, soybean tissue and orchardgrass several years after application (Price and Moschler, 1965). The availabilities of the various elements in the soil are changed by liming (Garrels and Christ, 1965). Zinc absorption by plants has been found to be a function of soil pH and not of calcium concentration in the soil (Wear, 1956; Wear and Patterson, 1965).

Calcium apparently alters the ratio of Na and K absorbed by plants. Overstreet *et al.* (1952) showed that Ca can increase or decrease K absorption, depending on the concentration of K supplied to the plant. Nielsen and Overstreet (1955) and Overstreet *et al.* (1952) have suggested that Ca may act as a co-factor in the utilization of the potassium-carrier complex, assuming K absorption involves the reaction $K^+ + HR \rightarrow KR + H^+$, where KR is the carrier complex, thus driving the reaction to the right. This suggestion would explain the stimulation of $K+$ absorption by liming the soil. Viets (1944) and Overstreet *et al.* (1955) have shown that Ca stimulated K uptake at some concentrations and inhibited uptake at other concentrations. This was explained by hypothesizing that Ca was taken up by two separate mechanisms, one of which is strongly inhibited by K and the other less so (Overstreet *et al.,* 1955).

Thomas and Krauss (1955) noted that Na accumulated in plants in proportion to the decrease in K in the rooting medium.

Fertilization Effects on Mg Content

Conroy and Lambe (1962) found that the Mg content of tomatoes decreased with P application to unlimed soil. These same workers found that liming increased soil Mg availability and increased uptake of Mg by tomatoes. Reith (1965) reported that application of K fertilizer just sufficient for optimum yield had practically no effect on Mg in the plant but excess K fertilizer tended to depress the Mg percentage. Bingham *et al.* (1958) found that excessive use of $Ca(H_2PO_4)_2$ fertilizer increased the Mg content of sour orange seedlings.

Effects on Micronutrient Content

Bingham and Garber (1960) found that excessive $Ca(H_2PO_4)_2$ applied to six California soils with a pH 6.0 increased Mn and Mo absorption by sour orange seedlings. Application of large quantities of $Ca(H_2PO_4)_2$ to alkaline soils reduced Mn and Mo in sour orange leaves to a level below the low $Ca(H_2PO_4)_2$ treatment.

Many workers have reported that P fertilization reduced Zn uptake by plants (Bingham and Barger, 1960; Bingham *et al.,* 1958; Olson *et al.,* 1965; Rogers and Wu, 1948; Wear and Patterson, 1965). The cause of the reduction in Zn uptake appeared to be a P-Zn physiological antagonism which probably occurred at the root surface (Olson *et al.,* 1965, Stukenholtz *et al.,* 1966). Phosphorus appeared to have a limited influence on Zn uptake where the soil exchange complex was relatively highly saturated with K (Olson *et al.,* 1965; Stukenholtz *et al.* 1966). Under conditions of low levels of soil P, K may even increase Zn concentration in some plants (Wear and Patterson, 1965). Boawn *et al.* (1954) reported that P application to a Washington soil had no effect on uptake by field beans of either applied or native soil Zn.

Other Nutritive Effects

Wiklander (1966) has shown that dilution of nutrient solutions from 0.1 to 0.0001 *M* strongly reduced the uptake of Ca, K, and Na by barley and pea plants, whereas only a slight influence was found on uptake of Mg.

All plants vary to some extent in their mineral element requirements, and every element in a nutrient media influences the other elements in relation to plant growth (Shutte, 1964).

MATERIALS AND METHODS

Site of the experiment was the Missouri Claypan Station near McCredie. The plot area had been in unimproved, ungrazed meadow for several years. Mexico silt loam was the predominant soil type in the area. Soil tests were made by standard Missouri soil testing procedures (Graham, 1959). Soil samples were taken before the green beans were planted in summer, 1965, and after the green bean harvest in September, 1965. The soil test data are summarized in Table 1.

A 2^3 x 3 factorially arranged randomized split plot design was used. The fertilizer treatments included three levels of N, two levels of lime, two levels of K, and two levels of P in all combinations with five replications. Carriers used were ammonium nitrate, 45 percent superphosphate, 60 percent muriate of potash, and calcium hydroxide.

The quantities of Ca, P, and K used for the spinach were different from those used for the green beans because of the change in soil test concentrations that resulted from the fertilization of the green beans prior to the spinach. The fertilizer treatments are given in Table 2. All fertilizers were broadcast and disced in after plowing.

Each plot was 10 x 20 feet. One commercial variety each of green beans and spinach was selected as listed in Table 3, with planting and harvesting dates. The vegetables were seeded in six rows per plot with Planet Junior seeders drawn by a small tractor. The green beans were thinned 15 days after planting to one plant every 3 inches. The spinach required no thinning after germination. Sprinkler irrigation was applied to the green beans on August 3 and 4, 1965. Natural rainfall was adequate thereafter. The spinach was irrigated only on April 2, 1966, to facilitate germination. Sevin insecticide was applied to the green beans; no other insecticide, fungicide, or herbicide was needed.

The green beans and spinach were hand harvested from measured rows within the 10 x 20 foot plots with due allowance for border effects. Harvests were made when growth on the most advanced plots was at a stage suitable for canning. Two grab samples were taken from bulk yields of each plot and washed with distilled wter. One sample was frozen for nitrate determinations and the other sample was dried at 65°C and was used in mineral analysis.

Nitrate was extracted from homogenized plant samples with hot 2 *N* KCl. Determinations of the NO_3-N in the extracts were made using reduction by De-

TABLE 1 - INITIAL (I) AND POST-GREENBEAN (PB) SOIL TEST VALUES--VEGETABLE EXPERIMENTAL SITE, CLAYPAN RESEARCH STATION, MCCREDIE, MO.

Treatment Code			OM %		P_2O_5 pp2m		K pp2m		Mg pp2m		Ca pp2m		pH water		pH salt		Exch H me/100g	
Ca	P	K	I*	PB*	I	PB	I	PB	I	PB	I	PB	I	PB	I	PB	I	PB
0	1	0	2.9	2.9	9	34	53	124	687	717	2575	2890	5.5	5.1	4.8	4.8	9.0	9.1
0	1	1	2.9	3.0	13	26	51	223	590	704	2658	3467	5.5	5.2	4.7	4.9	9.3	9.8
0	2	0	2.9	2.8	9	89	42	116	747	693	2767	2725	5.6	5.1	4.9	4.7	8.0	9.6
0	2	1	2.8	3.1	9	106	37	196	642	684	2375	3232	5.5	5.3	4.8	4.9	9.1	9.1
1	1	0	2.8	2.9	9	32	53	108	742	666	2710	4285	5.5	6.2	4.9	6.0	8.9	4.9
1	1	1	2.8	2.9	8	24	47	184	710	619	2467	4400	5.5	6.0	4.8	5.8	9.1	5.3
1	2	0	2.7	2.9	8	81	49	110	693	669	2510	4256	5.5	6.2	4.7	5.9	9.2	5.3
1	2	1	2.9	2.8	8	99	42	165	730	658	2610	4563	5.6	6.2	4.8	5.9	8.3	5.2

* I refers to initial soil samples (Spring 1966) and PB to the post greenbean soil samples (September 1965).

varda's alloy under live steam by a modification of the procedure presented by Bremner, 1965, and Brown and Smith, 1966.

The dried samples were ground in preparation for ashing. Two grams of the plant material were ashed at 500°C in fused silica crucibles. The white ash was then taken up in a minimum of 6 *N* redistilled HCl and the solution was transferred to 100 ml volumetric flasks and brought to volume with deionized water.

A Jarrell-Ash Model 82-516 atomic absorption, flame emission spectrometer with a Leeds and Northrup Speedomax-W recorder was used for Mg, Mn, and Zn determinations. Multi-element hollow cathode tubes were used as the source lamps.

Phosphorus was determined by the procedure outlined by Jackson (1958). Ca, K, and Na were determined on a Coleman Model 21 flame photometer. Standards for the flame photometer were made from $CaCO_3$ for Ca, KCl for K, and NaCl for Na. Standards for atomic absorption were made according to procedures published by McBride (1964). NO_3-N, Zn, and Mn were recorded as parts per million dry weight. Na, K, Ca, Mg and P were recorded as percent dry weight.

Statistical analyses of the data were made at the University of Missouri computer center and in general followed procedures outlined by Snedecor (1956).

TABLE 2 - TREATMENTS APPLIED TO MEXICO SILT LOAM FOR PRODUCTION OF GREEN BEANS AND SPINACH (1965-1966)

Treatment Code*	Element	Green beans+ lbs./A	Spinach+ lbs. A	Total	Carrier
Ca_0	Ca	0	0	0	unlimed
Ca_1	Ca	1650	4900	6550	$Ca(OH)_2$
P_1	P	22	10	32	45% Superphosphate
P_2	P	186	98	284	45% Superphosphate
K_0	K	0	0	0	untreated
K_1	K	138	83	221	60% KCl
N_1	N	100	100	200	NH_4NO_3
N_2	N	200	200	400	NH_4NO_3
N_3	N	300	300	600	NH_4NO_3

* The treatment codes will be utilized in all tables which present results.
\+ The green bean treatments were applied in July 1965 and were followed by the spinach treatments in March, 1966.

TABLE 3 - VARIETIES, PLANTING DATES, AND HARVESTING DATES OF GREEN BEANS AND SPINACH

Common Name	Varieties	Planting Date	Harvest Date
Snapbeans	Top Crop	July 26, 30, 1965	Sept. 20, 1965
Spinach	Viking	March 29, 1966	June 8, 1966

RESULTS AND DISCUSSION

Response to treatments in terms of plant composition was most striking on the spinach. Evaluation of the soil test results and plant composition data suggested that the time of incorporation of the fertilizers into the soil and the past history of the area were largely responsible for the smaller treatment response by the green beans. In the following discussion major emphasis will be placed upon statistically significant responses (Tables 4 and 5). Each vegetable will be discussed separately since the treatments applied resulted in different root environments in each case.

Spinach

The low rate of N (100 lbs./A) produced maximum yields of spinach when the soil pH was near neutrality and the high rates of K and P fertilizers had been applied (Table 6). On the unlimed plots the low yields were likely due to Mn toxicity.

The application of lime and N and K fertilizer increased NO_3-N concentration in the spinach (Table 7). Phosphorus fertilization had no significant direct effect on NO_3-N concentration but a strong Ca x P interaction on the NO_3-N level in the plant was observed (Table 4). Application of P to unlimed soil reduced spinach NO_3 concentration, but when the P was applied to limed soil, nitrate levels in the spinach increased.

This Ca x P interaction illustrates the complexity of the soil environment. Application of P increased yields, hence, when P was applied to unlimed soil, the reduced nitrate level in spinach may have been due to a combination of dilution in the plant and restricted soil nitrogen mineralization and availability due to the low pH. The reason for the increased nitrate content of the plants due to P application to limed soil is not clear. Possibly on the near neutral soil deficiency of some nutrient needed for nitrate reduction occurred. Alternatively, soil conditions may have been conducive to a nitrification rate in the soil in excess of reduction in the plant which, when combined with greater root proliferation due to the P treatment, resulted in net accumulation of nitrate.

Potassium fertilization, on the average, increased the nitrate content of the spinach by 900, 400, and 1400 ppm at rates of 100, 200, and 300 pounds of N per acre, respectively. This observation conflicts with the data reported by MacLeod (1965) who found that K fertilization tended to decrease NO_3-N in forages. One role of K in the plant is to maintain electrical neutrality within the plant; hence, as K was taken up by the plants, nitrate essentially accompanied the K. (This statement should not be construed to imply that the plant takes up KNO_3 as such but that an induction system may have been operative). Calculations based upon the yield and K and NO_3-N composition of the plant (Table 8) showed that at the low level of K fertilization, the ratio of me. K to NO_3-N taken up by the spinach was approximately 1:1 but when K fertilizer was ap-

TABLE 4 - SUMMARY OF THE ANALYSES OF VARIANCE OF THE EFFECTS OF FERTILITY TREATMENT ON YIELD AND ON SELECTED MINERAL ELEMENT CONTENTS OF SPINACH[a]

			Mean Squares						
Source	df	Yield	Nitrate $x10^{5}$	Ca $x10^{-3}$	K $x10^{-2}$	Mn $x10^{5}$	Zn $x10^{1}$	Mg $x10^{-4}$	Na $x10^{-2}$
Ca	1	6,452*	540*	2,567*	582*	80*	20,320*	957*	31*
P	1	11,142*	52	44	1,294*	12*	15,943*	517*	501*
K	1	12,280*	217*	30	5,691*	6*	39	2,142*	1,150*
N	2	342*	143*	151*	40	6*	777*	141	77
CaP	1	208*	543*	149*	7	10*	1,732*	14	2
CaK	1	231*	122	587*	7	6*	2	52	12
CaN	2	10	3	7	38	5*	231	175	31
PK	1	3,098*	2	199*	155*	15*	115	15	105*
PN	2	1*	21	9	46	3*	14	151	71
KN	2	153*	33	7	4	6*	75	130	3
CaPK	1	21	94	3	12	15*	39	568*	0
CaPN	2	2	15	45	2	4*	5	69	9
PKN	2	76*	17	120	3	5*	239	178	14
CaKN	2	11	9	7	15	6*	65	28	16
CaPKN	2	38	65	38	17	4*	177	3	10
Error	92	55	32	36	18	1	89	102	8

[a] In the table df refers to degrees of freedom, Ca, P, K and N to calcium, phosphorus, potassium and nitrogen, respectively.

* Significant at the 5% level.

TABLE 5 - SUMMARY OF THE ANALYSES OF VARIANCE OF THE EFFECTS OF FERTILITY TREATMENT ON YIELD AND ON SELECTED MINERAL ELEMENT CONTENTS OF GREEN BEANS[a]

Source	df	Yield	Mean Squares							
			NO_3-N	Ca $x10^{-2}$	P $x10^{-2}$	K $x10^{-2}$	Mg^{+} $x10^{-4}$	Na $x10^{-5}$	Zn	Mn
Replicate	4	748.82	837828	.59	.09	5.62	18.43	2.85	13.07	111.85
Ca	1	41.42	2448735*	3.89*	1.83*	.26	7.01	4.32*	240.83*	5200.83*
K	1	66.16	12282	.14	.18	547.84*	7.01	.05	320.13*	1254.53*
CaxK	1	7.96	93409	.01	.06	6.53	4.41	.75	1.20	313.63
Error a	12	146.96	56305	.15	.30	10.25	6.36	.45	18.44	241.30
P	1	266.71*	191361	12.03*	33.71*	11.41	648.68*	.12	50.70	168.03
CaxP	1	.20	48401	.74	.90*	.56	57.05*	.03	187.50*	34.13
PxK	1	90.65	17618	.19	.32	3.54	2.41	.16	22.53	20.83
CaxPxK	1	4.52	50103	.12	.18	5.04	3.01	.21	1.20	8.53
Error b	16	27.10	63302	.23	.15	2.59	5.38	.76	17.34	57.65
N	2	54.23	660234*	1.09*	.01	1.07	10.53	.52	3.73	631.43*
CaxN	2	2.98	44034	.20	.32	.47	4.13	1.03	14.03	173.23*
PxN	2	57.43	244017	.03	.03	.27	1.30	1.11	19.30	43.43
KxN	2	66.20	40413	.04	.22	3.36	4.13	1.32	6.43	101.03*
CaxPxN	2	3.95	1599	.07	.10	2.73	8.23	2.83*	103.60*	4.93
CaxKxN	2	5.13	734271*	.38	.35	5.10	6.93	1.75	4.90	182.53*
PxKxN	2	15.11	772534*	.31	.39	4.89	4.23	.21	3.63	21.23
CaxPxKxN	2	7.44	92061	.29	.03	.36	15.43*	1.00	0.70	12.03
Error c	64	35.57	80911	.19	.16	3.14	4.33	.72	25.04	23.30
Total	119	-	-	-	-	-	-	-	-	-

[a] In the table df refers to degrees of freedom, Ca, P, K and N to calcium, phosphorus, potassium and nitrogen, respectively.

* Denotes significance at the 5% level by standard F-test.

TABLE 6 - EFFECT OF SOIL TREATMENT ON YIELD OF FRESH SPINACH

Treatment Codes			Nitrogen Treatment lbs/A			
Ca	P	K	100	200	300	Mean
			(lbs./100 foot row)			
0	1	0	5.3	1.9	3.9	3.7
0	1	1	14.7	11.0	6.3	10.7
0	2	0	9.3	9.2	11.0	9.8
0	2	1	45.2	36.8	32.9	38.3
1	1	0	15.3	13.3	9.2	12.6
1	1	1	29.4	29.1	24.3	27.6
1	2	0	26.1	23.9	25.5	25.2
1	2	1	63.1	59.5	50.1	57.6
	Mean		26.1	23.1	20.4	23.2

* The codes correspond to those given in Table 2.

TABLE 7 - AVERAGE COMPOSITION OF SPINACH GROWN UNDER VARIOUS LEVELS OF SOIL FERTILITY (DRY WEIGHT BASIS)

Treatment Code*	NO_3-N ppm	K %	Ca %	Mg %	Na %	Mn ppm	Zn ppm
$Ca_0P_1K_0N_1$	5024	0.98	0.72	1.15	1.44	471	213
$Ca_0P_1K_0N_2$	4187	0.76	0.71	1.11	1.44	509	203
$Ca_0P_1K_0N_3$	4694	1.26	0.67	1.00	1.04	417	185
$Ca_0P_1K_1N_1$	4605	2.67	0.72	0.97	0.70	468	176
$Ca_0P_1K_1N_2$	4685	2.21	0.86	0.95	0.71	864	211
$Ca_0P_1K_1N_3$	7200	2.66	1.08	0.96	0.43	2132	257
$Ca_0P_2K_0N_1$	3133	0.37	0.54	1.00	1.77	343	95
$Ca_0P_2K_0N_2$	4636	0.62	0.73	1.02	1.57	774	124
$Ca_0P_2K_0N_3$	5289	0.69	0.72	0.98	1.16	472	122
$Ca_0P_2K_1N_1$	3716	1.63	0.68	0.99	1.34	331	101
$Ca_0P_2K_1N_2$	4023	1.65	0.79	1.00	1.23	332	91
$Ca_0P_2K_1N_3$	4008	1.90	0.62	0.95	0.91	446	130
$Ca_1P_1K_0N_1$	1254	1.56	0.92	1.03	1.36	141	93
$Ca_1P_1K_0N_2$	2175	1.20	1.00	1.05	1.47	118	112
$Ca_1P_1K_0N_3$	2180	1.27	1.07	0.93	1.50	130	111
$Ca_1P_1K_1N_1$	2856	3.33	0.88	0.90	0.50	117	89
$Ca_1P_1K_1N_2$	2946	2.87	1.00	0.99	0.52	149	117
$Ca_1P_1K_1N_3$	3360	3.10	1.03	0.92	0.65	131	109
$Ca_1P_2K_0N_1$	2334	1.12	1.05	0.96	1.66	102	48
$Ca_1P_2K_0N_2$	3747	0.84	1.22	0.98	1.74	120	66
$Ca_1P_2K_0N_3$	3390	0.93	1.27	1.04	1.68	128	63
$Ca_1P_3K_1N_1$	4313	2.13	0.89	0.80	1.46	120	47
$Ca_1P_2K_1N_2$	4529	2.21	0.87	0.89	1.36	109	53
$Ca_1P_2K_1N_3$	6506	1.97	0.98	0.88	0.76	119	60

* Treatment codes correspond to those given in Table 1.

plied the K:NO_3-N ratio in the plant approximated 2:1. This ratio in either case was highest at the nitrogen rate of 100 pounds per acre and decreased with increasing rates of nitrogen.

These data indicate that high K in the rooting medium may result in increased K uptake by the plant which could induce the uptake of nitrate. The data also indicate that increased levels of nitrate in the medium caused increased uptake of nitrate. The specific cause of increased nitrate in spinach due to application of K is not clear; it has been observed in solution studies previously but not in field studies (Wright and Davison 1964).

TABLE 8 - RATIO OF MILLIEQUIVALENTS OF K TO MILLIEQUIVALENTS OF NO3-N TAKEN UP PER 100 FOOT ROW OF SPINACH

Potassium Treatment*	Nitrogen Treatment (lbs/A)			Mean
	100	200	300	
K_0	1.24	.83	.96	1.01
K_1	2.25	1.89	1.64	1.96

* Codes correspond to Table 2.

The Ca concentration in spinach was influenced by all treatments in a complex manner (Table 9). The main effects of lime and N were statistically significant but so were several interactions; e.g., Ca x P, Ca x K, Ca x N and P x K x N (Table 4). The interaction of K with lime is interesting. The data indicate that K fertilization increased Ca concentration in the plants grown on unlimed soils, but decreased Ca concentration in plants grow on limed soil. The complementary ion effect in the soil and dilution within the plant due to increased growth are operative simultaneously; hence, the Ca concentration of the spinach grown on any given treatment is the net result of these two phenomena.

Phosphorus fertilizer effects on the Ca concentration also depended upon whether the soil was limed or unlimed. On limed soil P treatment increased the Ca concentration of the spinach, but on unlimed soil P addition decreased Ca concentration.

TABLE 9 - AVERAGE CALCIUM CONCENTRATION OF SPINACH GROWN UNDER DIFFERENT FERTILITY LEVELS (% DRY WEIGHT)

Treatment Codes	Treatment Codes*					
	K_0			K_1		
	N_1	N_2	N_3	N_1	N_2	N_3
Ca_0P_1	0.72	0.71	0.67	0.72	0.86	1.08
Ca_0P_2	0.54	0 73	0.72	0.68	0.79	0.62
Ca_1P_1	0.92	1.00	1.07	0.88	1.00	1.03
Ca_1P_2	1.05	1.22	1.27	0.89	0.87	0.98

* Codes correspond to Table 2.

The effect of P on Ca concentration of spinach grown on unlimed soil may be explained by dilution due to increased yield (Tables 6 and 9) but this explanation could not apply to spinach grown on limed soil. The increase in Ca concentration of spinach due to P on limed soil must have been associated with increased vigor of the plants (and perhaps greater soil contact by the roots) and the fact that the level of Ca in the soil was sufficiently high to offset any dilution in the plant resulting from the maximum growth of the spinach grown on the Ca_1P_2 plots.

Nitrogen fertilization tended to increase the Ca concentration of spinach on both limed and unlimed soil except for the N_3 treatment on unlimed soil. On unlimed soil, the N_3 treatment gave a lower Ca concentration than the N_1 treatment. These effects of N must be associated with increased H ion activity in the soil resulting from nitrification.

The K, lime, and P treatments had significant direct effects upon the K concentration of the spinach (Table 4). The K fertilizer effect was purely a matter of availability in the soil. Phosphorus fertilization reduced the K concentration in the spinach both at low and high soil K levels; this is likely a dilution effect on K because of increased yield. Lime increased the K concentration in the spinach, perhaps as a result of the complementary ion effect and operation of the carrier concept as proposed by Overstreet and co-workers (Nielson and Overstreet, 1955, Overstreet *et al.,* 1952).

All main effects and interactions were significant for the Mn concentration (Table 4). Mn toxicity symptoms were observed on unlimed plots. It is likely that as various treatments were applied, the availability of soil Mn, coupled with growth changes and consequent changes in dilution, were responsible for the many interactions. Also, the wet weather probably influenced Mn availability. Since P and K deficiency symptoms were also observed in plots where those elements were not applied to the soil, it was apparent that many degrees of deficiency of P and K and toxicity of Mn could have been created by the various treatment combinations. Schutte (1964) aptly described this situation: "...it must be appreciated that not all deficient plants show (deficiency symptoms)."

The Mn concentration in the spinach was decreased by lime, P, and K and increased by the N treatments. The magnitude of the decrease or increase in the Mn concentration due to treatment depended, of course, upon the combination of treatments. Liming caused the greatest decrease in Mn concentration in the spinach leaves due to its raising soil pH. The increase in Mn concentration due to N treatment was probably due to increased soil acidity resulting from nitrification of the ammonia from the applied NH_4NO_3. The soil which received 300 pounds of N per acre had a soil pH .2 to .3 unit lower than the soil which received 100 pounds of N.

Increasing rates of N increased the Zn concentration of the spinach, probably due to the effect on soil pH (Tables 4 and 7). Lime and P both decreased Zn concentration but the Ca x P interaction was significant. Figure 1 shows the magnitude of these effects. Phosphorus has been shown to decrease the solubility of Zn in the soil and to affect Zn uptake (Bingham and Garber, 1960; Stukenholtz *et al.*, 1966). Potassium fertilization had no effect upon Zn concentration in the spinach.

Lime, P, and K additions had statistically significant influences on the Mg concentration in the spinach leaves, but the third order interaction of these three treatments was also significant (Table 4). Figure 2 illustrates these effects. Potassium application strongly decreased the Mg concentration of the spinach leaves; this effect was attributed to a combined influence of the complementary ion effect and dilution within the plant as a result of increased growth due to K treatment. Due to yield increases obtained with K, the decrease of the Mg percentage in the leaves appears to conflict with results given by Reith (1965).

As would be expected, K decreased the Na concentration in the leaves; however, there were significant second order interactions, i.e. Ca x N, P x K and P x N (Table 4). It is suggested that these interactions arose due to the effect of lime and P on growth.

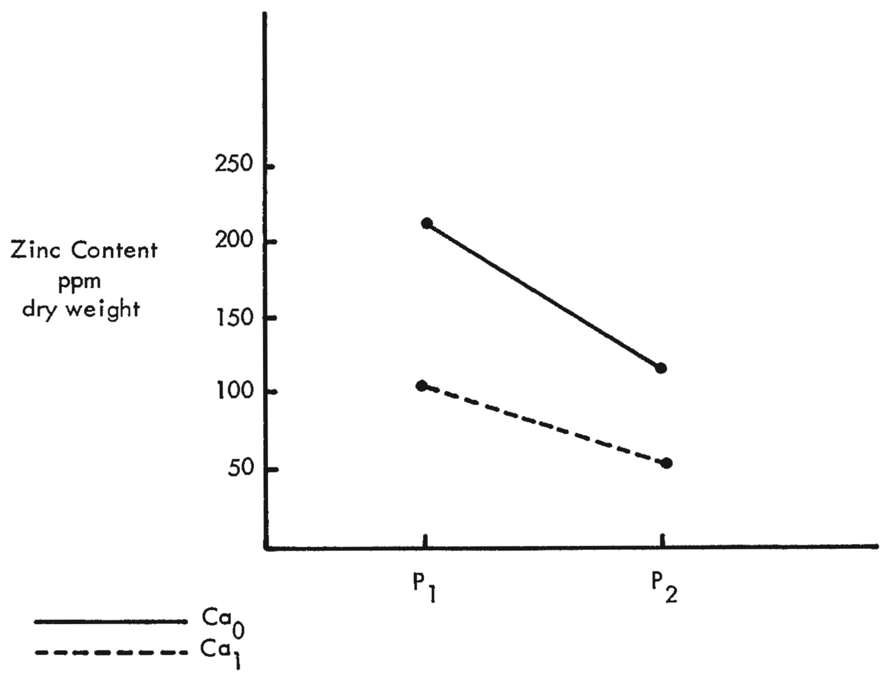

Fig. 1—Effect of Ca and P soil treatments on the Zn content of spinach leaves.

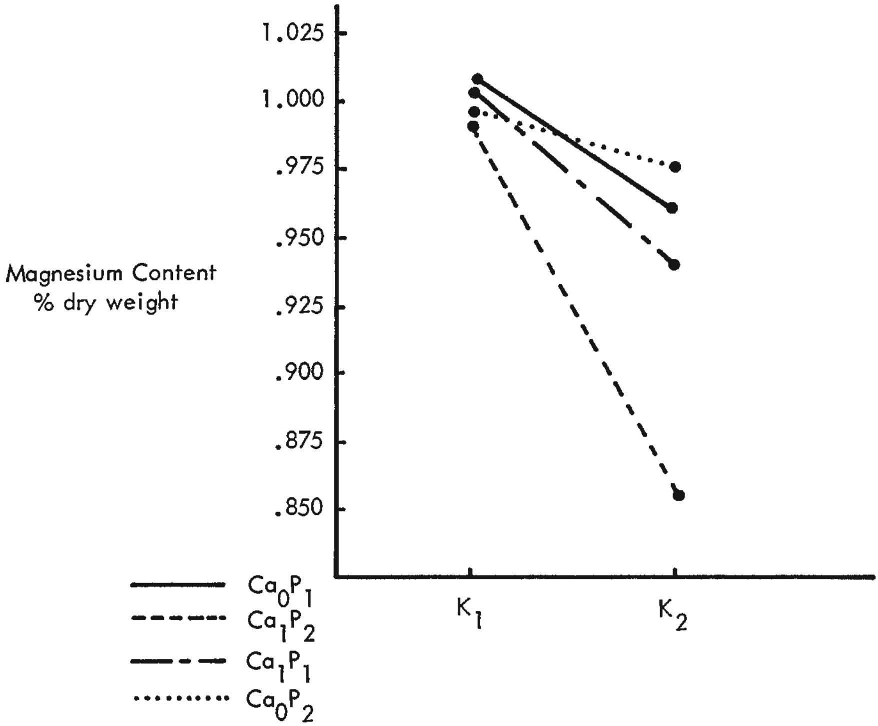

Fig. 2—Effect of Ca, P, and K soil treatments on the Mg content of spinach leaves.

Green Beans

The yield of green beans was not greatly affected by any treatment. Growth conditions appeared optimum. The land was plowed and laid fallow for a month prior to seeding, which apparently accomplished several things contributing to the lack of treatment response. Plowing killed the growing vegetation, thus reducing competition for soil nutrients. The warm weather, coupled with optimum but not excessive rainfall and the open nature of the fallow soil resulted in mineralization of soil N and P as well as oxidation of Mn. Thus, at the time of planting, a supply of available nutrients had accumulated. The average yield of green bean pods was 36.2 pounds of fresh pods per 100 feet of row. The coefficient of variability was 17 percent which indicated considerable variation unaccounted for.

The 200 pounds of N per acre caused the greatest NO_3-N accumulation in the green bean pods (Tables 10 and 11). The response to N fertilizer as measured by NO_3-N accumulation in the bean pods is similar to the dual peaks observed with increasing rates of nitrogen in several vegetables by Brown and

TABLE 10 - AVERAGE COMPOSITION OF GREEN BEANS PRODUCED UNDER VARIOUS LEVELS OF SOIL FERTILITY (DRY WEIGHT BASIS)

Treatment Code*	NO_3-N ppm	P %	K %	Ca %	Mg %	Mn ppm	Zn ppm
$Ca_0P_1K_0N_1$	1717	.32	1.78	.40	.12	41	31
$Ca_0P_1K_0N_2$	1767	.26	1.61	.43	.16	45	28
$Ca_0P_1K_0N_3$	1975	.29	1.82	.43	.11	48	29
$Ca_0P_1K_1N_1$	1268	.29	1.89	.39	.14	38	34
$Ca_0P_2K_1N_2$	1895	.30	2.04	.41	.13	42	33
$Ca_0P_1K_1N_3$	1885	.27	1.80	.43	.13	55	32
$Ca_0P_2K_0N_1$	1347	.41	1.59	.29	.07	36	30
$Ca_0P_2K_0N_2$	3094	.42	1.87	.33	.08	45	32
$Ca_0P_2K_0N_3$	2165	.39	1.77	.36	.08	43	33
$Ca_0P_2K_1N_1$	1951	.43	2.06	.33	.07	43	37
$Ca_0P_2K_1N_2$	2194	.42	1.91	.34	.08	44	35
$Ca_0P_2K_1N_3$	1228	.40	1.99	.36	.09	55	39
$Ca_1P_1K_0N_1$	1875	.31	1.40	.44	.13	34	25
$Ca_1P_1K_0N_2$	2318	.34	1.68	.41	.12	37	35
$Ca_1P_1K_0N_3$	1915	.36	1.62	.44	.12	37	32
$Ca_1P_1K_1N_1$	2129	.33	1.93	.39	.11	35	27
$Ca_1P_1K_1N_2$	1926	.33	2.03	.48	.16	41	30
$Ca_1P_1K_1N_3$	2260	.32	2.00	.44	.16	42	34
$Ca_1P_2K_0N_1$	1804	.38	1.70	.38	.09	32	31
$Ca_1P_2K_0N_2$	2310	.42	1.56	.39	.09	36	27
$Ca_1P_2K_0N_3$	1991	.42	1.68	.38	.10	36	28
$Ca_1P_2K_1N_1$	2068	.45	2.01	.38	.09	35	32
$Ca_1P_2K_1N_2$	2317	.42	1.94	.39	.10	40	32
$Ca_1P_2K_1N_3$	1802	.43	2.07	.40	.10	36	32

* Codes correspond to Table 2.

TABLE 11 - AVERAGE NO_3-N CONCENTRATION IN GREEN BEANS (DRY WEIGHT BASIS)

Treatment Code*		Nitrogen Code			
Ca	K	N_1 ppm	N_2 ppm	N_3 ppm	Mean ppm
0	0	1532	2430	2070	2011
0	1	1610	2044	1556	1737
1	0	1840	2314	1953	2036
1	1	2098	2122	2031	2084
Mean		1770	2218	1902	

* Codes correspond to Table 2.

Smith (1966, 1967). There was no specific explanation for this phenomena. Liming the soil caused a significant increase in pod NO_3-N, probably due to improved nitrification as a consequence of the higher pH produced by the lime. The significant Ca x K x N interaction suggests that the mechanism of NO_3-N accumulation in the pods is more complex than a direct effect of lime or N fertilizer (Table 11).

Nitrogen fertilization and liming significantly increased and P fertilization decreased the Ca concentration of the bean pods (Table 10). There were no significant interactions between treatments. Liming could increase Ca availability to the plant by mass action whereas P could have caused formation of insoluble calcium phosphates which would reduce the supply of available Ca. One explanation of the N effect is that increased H ion activity due to nitrification offset the P effect.

The carrier concept as proposed by Overstreet and co-workers (Nielson and Overstreet, 1955; Overstreet *et al.,* 1952) which related Ca and K in the ion uptake mechanism(s) is of value in explaining the significant interaction among lime and K fertilizer and the K concentration in the pods (Table 12). Liming suppressed the K concentration in the bean pods where K fertilizer was not applied but increased the K concentration where K was added to the soil. Thus, the stimulating effect of Ca on K uptake appeared to depend upon the level of available K in the soil as inferred by Overstreet *et al.* (1952), and Viets (1944). Potassium fertilization directly increased the K concentration in the bean pods. Some reservations should be expressed concerning the effects of lime and K fertilizer on the K concentration in the pods because the fourth order interaction (Ca x P x K x N) was significant at the 5 percent level (Table 5).

TABLE 12 - AVERAGE PERCENTAGE K IN GREENBEANS GROWN AT DIFFERENT LEVELS OF LIME AND POTASSIUM IN THE SOIL (DRY WEIGHT BASIS)*

Treatment Code	Treatment Code	
	K_0 %	K_1 %
Ca_0	1.74	1.91
Ca_1	1.61	1.99

* Codes correspond to Table 2.

Although no Mn toxicities were noted in the snapbeans, the treatments significantly affected the Mn concentration of the bean pods (Table 5). The Ca x N interaction was significant as were the main effects of lime (Ca), N, and K (Table 13).

TABLE 13 - INFLUENCE OF SOIL TREATMENTS ON MN CONCENTRATION OF GREEN BEANS (DRY WEIGHT BASIS)

(a) Treatment Code*	Treatment Code* N_1	N_2	N_3
Ca_0	.39 ppm	.44 ppm	.50 ppm
Ca_1	.34	.38	.38

(b)	Treatment Code*	ppm Mn
	K_0	.39
	K_1	.42

* Codes correspond to Table 2.

Potassium increase in the soil due to treatment probably increased the availability of Mn in the soil through a mass action effect on exchangeable Mn and (or) through the effect of the chloride ion acting with hydrogen ions. The Ca x N interaction was probably related to pH changes in the soil. Nitrification of the ammonium ion in ammonium nitrate increased the hydrogen ion activity in the soil; this activity appeared to have greatest effect in the absence of lime, since lime would tend to neutralize hydrogen which in turn would lower the availability of Mn in the soil.

One would expect the effects on Zn to be similar to those on Mn; however, only lime and K treatments significantly influenced the Zn concentration in the bean pods. Liming decreased the Zn concentration in the pods from 33 ppm to 30 ppm, apparently due to decreased Zn availability resulting from an increase in soil pH (Wear, 1956). The application of K increased the Zn concentration in the pods from 30 ppm to 33 ppm, due probably to the same mechanism(s) which operated with Mn.

Phosphorus addition decreased the Mg concentration in the bean pods from 0.13 percent to 0.09 percent. The effect of P treatment on Mg probably resulted from soil reactions between phosphate and Mg ions resulting in reduced Mg availability.

Sodium concentration in the pods varied considerably and no significant treatment effect was noted.

As noted in Table 5, both Ca and P soil treatments significantly influenced the P composition of the green beans. On unlimed soil those grown at the low P treatment contained 0.29 percent P while those grown at the high P treatment contained 0.41 percent P. Liming the soil tended to increase the P composition of the beans at the low P soil level more than at the high P soil level (actual concentration was 0.33 percent P at the Ca_1P_1 level and 0.42 percent at the Ca_1P_2 level) hence the significant Ca x P interaction (Table 5). These effects and one interaction may have been related to root vigor since yield was not affected.

SUMMARY

Spinach

The fact that maximum yields were attained with the low rate of nitrogen indicates that *under the conditions of this study* the optimum level of nitrogen fertilization was at or below 100 pounds per acre when lime, P, and K supplies in the soil were adequate.

Both the P and lime status of the soil affected the NO_3-N level of the spinach. Phosphorus decreased the NO_3-N concentration in spinach grown on unlimed soil but increased NO_3-N concentration of the spinach leaves on limed soil.

The Ca concentration of spinach leaves was the result of many interacting fertility factors. K fertilization increased and P fertilization decreased the Ca concentration of spinach on unlimed soil; opposite effects were found on limed soil.

The carrier concept was offered as an explanation for the increase in K concentration of the spinach grown on limed soil as compared with unlimed soil. Phosphorus, by increasing spinach growth, tended to decrease the K concentration in the leaves.

Mn concentration of the spinach leaves was influenced by all fertility practices. The absolute effect of any one treatment was affected by the level of all other treatments. Soil moisture, as a result of the wet growing weather and changes in soil pH, had profound influence on availability of Mn in the soil.

Mg concentration in the spinach was reduced by application of K fertilizer. The cause of this reduction was thought to be a combination of dilution by the growth increase caused by K and the operation of the complementary ion effect in the soil with K and Mg as the functioning cations.

Both P and lime additions decreased the Zn concentration of spinach but the magnitude of the effect of either P or lime depended on the level of the other. Increasing rates of N fertilization increased the Zn concentration of the spinach through a decrease of soil pH by nitrification of the ammonia from the NH_4NO_3 source.

Green Beans

Yield of green beans was not greatly affected by any treatment. The lack of yield response was probably due to pre-planting cultural practices since soil tests indicated low levels of Ca, P, and K in the soil.

Maximum NO_3-N concentration in the bean pods was achieved with 200 pounds of N per acre. NO_3-N accumulation in the pods was shown to be more complex than a response to N fertilizer alone.

The K concentration in the beans was influenced by both lime and K fertilizer. Liming the soil reduced K concentration on the K_2 treatment. These results are evidence of the operation of the enhancing effect of Ca on K uptake where K is not limiting in the soil.

Mn concentration in the beans was increased by increasing rates of N fertilizer on unlimed soil but was only slightly affected by N on limed soil. K fertilization increased the Mn concentration in the beans.

The Zn concentration in the beans was decreased by liming and increased by K fertilization.

LITERATURE CITED

Beeson, K. C., L. Gray and K. C. Hamner, 1948. Absorption of mineral elements by forage plants: II. The effect of fertilizer elements and liming materials on the content of mineral nutrients in soybean leaves. J. Amer. Soc. Agron. 44:553-562.

Bingham, F. T. and M. J. Garber, 1960. Solubility and availability of micronutrients in relation to phosphorus fertilization. Soil Sci. Soc. Amer. Proc. 24:209-213.

Bingham, F. T., J. P. Martin and J. A. Chastain, 1958. Effects of phosphorus fertilization of California soils on minor element nutrition of citrus Soil Sci. 86:24-31

Boawn, L. C., F. G. Viets, Jr. and C. L. Crawford. 1954. Effect of phosphate fertilizers on zinc nutrition of field beans. Soil Sci. 78:1-7

Bremner, J M. 1965. Inorganic forms of nitrogen Chapter 84 *in* C. A. Black (ed) Methods of soil analysis, Part 2. Amer. Soc. of Agron. Inc. Madison, Wisc.

Brown, J. R. and G. E. Smith. 1967. Nitrate accumulation in vegetable crops as influenced by soil fertility practices. Missouri Agr. Exp. Sta. Res. Bull. 920, 44 pp.

Brown, J. R. and G. E. Smith. 1966. Soil Fertilization and nitrate accumulation in vegetables. Agron. J. 58:209-212.

Conroy, E. and J. C. D. Lambe. 1962. A phosphorus magnesium interaction in tomato nutrition. Nature 194:500.

Ehrendorfer, K. 1964. Ein Beitrag zur Kenntnis des Einflus der Kaliumdungung auf Substanzbildung und Mahrstoffaufnahme bei Spinat *(Spinacea oleracea L.)* Bodenkultur 15: 105-118.

Garrels, R. M. and C. L. Christ. 1965. Solutions, minerals and equilibria. Harper and Row, New York. 450 p.

Graham, E. R. 1959. An explanation of theory and methods of soil testing. Missouri Agric. Exp. Sta. Bull. 734. 20 p.

Grunes, D. L. 1959. Effects of nitrogen on the availability of soil and fertilizer phosphorus to plants. Advances in Agron. 11:369-396.

Hanway, J. J. and A. J. Englehorn. 1958. Nitrate accumulation in some Iowa crop plants. Agron. J. 50:331-334.

Jackson, M. L. 1958. Soil Chemical analysis. Prentice-Hall, Inc. Englewood Cliffs, N. J. 498 p.

Kresge, C. B. and S. E. Younts. 1963. Response of orchardgrass to potassium and nitrogen fertilization on a Wickham silt loam. Agron. J. 55:161-164.

Lunin, J., M. H. Gallatin and A. R. Batchhelder. 1964. Interactive effects of soil fertility and salinity on the growth and composition of beans. Proc. Amer. Soc. Hort. Sci. 85:350-360.

MacLead, L. B. 1965. Effect of nitrogen and potassium on yield and chemical composition of alfalfa, bromegrass, orchardgrass and timothy grown as pure species. Agron. J. 57:261-266.

McBride, C. H. 1964. Determination of minor nutrients in fertilizers by atomic absorption spectrophotometry. Atomic Absorption Newsletter 3:144-159.

Nielson, T. R. and R. Overstreet. 1955. A study of the role of the hydrogen ion in the mechanism of potassium absorption by excised barley roots. Plant Physiol. 30:303-309.

Olson, R. A., D. D. Stukenholtz, and C. A. Hooker, 1965. Phosphorus zinc relations in corn and sorghum production. Better crops with Plant Food 49:No1:19-24.

Overstreet, R., L. Jacobson and R. Handley, 1952. The effect of calcium on the absorption of potassium by barley roots. Plant Physiol. 27:583-590.

Perez, C. B., Jr., and C. D. Story. 1960. The effect of nitrate in nitrogen-fertilized hays on fermentation *in vitro*. J. Anim. Sci. 19:1311.

Price, N. O. and W. W. Moschler. 1965. Plant uptake of minerals, effect of residual lime in soil on minor elements in plants. J. Agric. Food Chem. 13:163-165.

Reith, J. W. S. 1965. Mineral composition of crops. N.A.A.S. Quarterly Rev. 68:150-156.

Rogers, L. H and C. Wu. 1948. Zinc uptake by oats as influenced by application of lime and phosphate. J. Amer. Soc. Agron. 40:563-566.

Schutte, K. H. 1964. The biology of the trace elements. J. B. Lippincott Company, Philadelphia. 228 p.

Smith, D. and J. M. Sund. 1965. Influence of stage of growth and soil nitrogen on nitrate content of herbage on alfalfa, red clover, ladino clover, trifoil, and bromegrass. J. Agric. Food Chem. 13:81-84.

Snedecor, G. W. 1956. Statistical methods, Iowa State College Press, Ames. 534 p.

Stukenholtz, D. D., R. J. Olsen, G. Gogan and R. A. Olson. 1966. On the mechanism of phosphorus-zinc interaction in corn nutrition. Soil Sci. Soc. Amer. Proc. 30:759-763.

Thomas, W. H. and R. W. Krauss. 1955. Nitrogen metabolism in Scehedesmus as affected by environmental changes. Plant Physiol. 30:113-122.

Tisdale, S. L. and W. L. Nelson. 1966. Soil fertility and fertilizers. 2d ed. The Macmillan Company, New York, 694 p.

Tserling, W. V. 1965. Study of the role of nutrients in the formation of yields as a basis of plant diagnosis. Soviet Soil Sci. 1965:8:929-940.

Viets, F. G., Jr. 1944. Calcium and other polyvalent cations as accelerators of ion accumulation by excised barley roots. Plant Physiol. 19:466-480.

Wear, J. I. 1956. Effect of soil pH and calcium on the uptake of zinc by plants. Soil Sci. 81:311-315.

Wear, J. I. and R. M. Patterson. 1965. Potassium fertilization and phosphorus-zinc relationship. Crops and Soils 18:11.

Wiklander, L. 1966. Nutrient uptake by plants as influenced by concentrations. Lantbrukshogekolans Annaler 32:87-92.

Wright, M. J. and K. L. Davison. 1964. Nitrate accumulation in crops and nitrate poisoning in animals. Advances in Agron. 16:197-247.