Effect of Phosphorus and Atrazine on Mineral Composition of Soybeans

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In a growth chamber study, addition of phosphorus to the soil increased the sensitivity of soybeans to sublethal concentrations of atrazine. Dry matter production and the accumulation of phosphorus, calcium, barium, sodium, and cobalt by soybean shoots were affected by both phosphorus and atrazine treatments; accumulation of manganese by phosphorus treatments; and accumulation of iron, zinc, molybdenum, and silicon by atrazine treatments. A phosphorus-atrazine interaction influenced the shoot content of magnesium, manganese, boron, and barium.

Numerous reports (Adams, 1965; Bingham and Upchurch, 1959; Rakitin and Potapova, 1959; Upchurch, 1963; Upchurch *et al.*, 1963) have suggested that various herbicides influence the uptake of phosphorus and its metabolism by plants. Upchurch *et al.* (1963) and Adams (1965) indicated that simazine [2-chloro-4, 6-bis-(ethylamino)-*s*-triazine] and phosphorus might interact to increase the sensitivity of soybeans (*Glycine max L.*) to simazine. Howell and coworkers (Bernard and Howell, 1964; Dunphy *et al.*, 1966; Howell, 1964; Howell and Bernard, 1961) and Fletcher and Kurtz (1964) demonstrated that certain varieties of soybeans, including the variety Chippewa, are sensitive to high concentrations of phosphorus. The latter workers found a major effect from phosphorus on the uptake of several mineral elements.

The present study was conducted to evaluate the effects of phosphorus and/or atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino-*s*-triazine] upon the growth and accumulation of mineral elements by soybean seedlings.

MATERIALS AND METHODS

The plants used were grown in growth chambers under controlled light and temperature conditions. The temperature was held at 21° ± 2° C. during 16 hours of light with an intensity of 2000 foot-candles and at 16° ± 1° C. during 8 hours of darkness. Samples of a Foreman clay loam, Buse clay loam, and Webster clay loam were used. Pertinent soil test data are given in Table I.

Wax-coated cardboard quart containers were used as pots. Each container was filled with 800 grams of thoroughly mixed soil-fertilizer-herbicide mixture (soil air-dried). Reagent grade monobasic calcium phosphate [$Ca(H_2PO_4)_2XH_2O$] was applied at rates of 0, 325, 650, 975, and 1300 mg. per pot. Atrazine was applied to the soil in 95% ethanol solutions at rates of 0, 0.2, and 0.4 p.p.m. Equivalent amounts of alcohol were added to each pot (including checks) and allowed to evaporate before seeding. Pots were seeded with 20 soybean seeds, variety Chippewa. Duplicate pots were prepared for each treatment. A randomized block design was used. Pots were watered at the surface with deionized water to approximately field capacity every second or third day, as needed.

Shoots were harvested 35 days after the date of planting and dry weights determined. The dry plant material was sent to Ohio State University to be analyzed by emission spectrography for 16 mineral elements: potassium, phosphorus, calcium, magnesium, strontium, manganese, iron, copper, cobalt, molybdenum, boron, aluminum, silicon, sodium, zinc, and barium.

The data were submitted to an analysis of variance to determine the main and interaction effects of treatments on dry matter produced, the concentration of each element in the dry plant material, and the total accumulation of each element.

RESULTS AND DISCUSSION

Data for elements that were consistently affected by the treatments and/or of major concern in plant nutrition are re-

Table I. Soil Test Data for Soil Samples Used in Studying Phosphorus-Atrazine Interactions[a]

Soil Sample	pH	Organic Matter, %	Extractable P, P.P.M.	Exchangeable K, P.P.M.	Cation Exchange Capacity, Meq./100 Grams	Clay, %
Forman clay loam[b]	7.1	5.3	6	175	34.3	40.1
Buse clay loam[b]	7.6	3.9	4.5	145	28.4	43.4
Webster clay loam	5.8	7.1	20	130	37.6	38.0

[a] Organic matter determined by Walkley-Black procedure; phosphorus by Bray No. 1 extraction; exchangeable K by normal ammonium acetate extraction; cation exchange capacity by saturation with normal ammonium acetate and extraction with 10% sodium chloride; clay content by Bouyoucos method.
[b] These soils are found in association and are currently mapped as Forman and Buse. However, the clay content of both exceeds the maximum limits established and cannot be considered typical.

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ported in Table II. Results from the analyses for copper, aluminum, sodium, strontium, and cobalt have been omitted. Both phosphorus and atrazine produced significant differences in accumulation of sodium and cobalt, but results were so variable that they could not be interpreted with confidence. The remaining three elements were not significantly affected by the phosphorus treatments or by an interaction between phosphorus and atrazine. Table III gives the statistical interpretation of these data.

Figure 1 shows the effects of the atrazine-phosphorus interaction on the growth of soybeans in the Forman clay loam soil. In this study and later unpublished work, the triazine-phosphorus interaction has been most readily demonstrated with calcareous soil samples of good fertility. Such dramatic results as shown in Figure 1 were not always obtained. The interaction effect of atrazine and phosphorus significantly reduced dry matter production by soybeans grown in the Forman clay loam sample, but not with the Buse or Webster clay

Table II. Mineral Composition of Soybean Shoots Grown on Three Soil Samples with Three Atrazine and Five Phosphorus Treatments

$Ca(H_2PO_4)_2$ Applied, P.P.M., P.	Growth Dry Wt., G./Pot	P, %	K, %	Ca, %	Mg, %	Fe, P.P.M.	Mn, P.P.M.	B, P.P.M.	Zn, P.P.M.	Mo, P.P.M.	Si, P.P.M.	Ba, P.P.M.
Forman Clay Loam, No Atrazine												
0	2.19	0.22	1.24	1.42	0.43	49	46	49	26	0.35	0.06	20
50	5.04	0.22	1.08	1.68	0.47	46	46	46	22	0.36	0.06	22
100	5.00	0.34	1.11	1.69	0.48	46	42	46	22	0.40	0.08	24
150	4.85	0.32	1.01	1.51	0.44	45	40	45	16	0.33	0.08	22
200	5.04	0.41	1.12	1.68	0.45	44	50	44	20	0.44	0.13	23
0.2 P.P.M. Atrazine												
0	4.06	0.23	1.16	1.29	0.37	48	44	48	24	0.22	0.07	16
50	4.59	0.30	1.25	1.45	0.33	46	34	46	30	0.22	0.08	12
100	4.43	0.35	1.42	1.78	0.50	52	48	52	26	0.41	0.12	26
150	3.89	0.46	1.69	1.74	0.52	46	48	46	27	0.42	0.10	25
200	4.13	0.50	1.42	1.80	0.51	45	46	45	24	0.41	0.12	26
0.4 P.P.M. Atrazine												
0	2.70	0.41	2.12	1.80	0.56	64	63	64	40	0.60	0.12	30
50	2.42	0.45	2.19	1.90	0.60	58	54	58	37	0.67	0.17	32
100	2.14	0.55	2.58	1.89	0.55	58	40	58	37	0.68	0.20	36
150	1.83	0.61	2.81	1.88	0.56	60	42	60	38	0.69	0.20	37
200	1.43	0.64	2.76	1.97	0.52	56	50	56	36	0.72	0.14	38
Buse Clay Loam, No Atrazine												
0	7.86	0.16	1.34	1.26	0.42	50	42	31	18	0.36	0.13	22
50	7.40	0.21	1.44	1.50	0.48	46	40	34	16	0.37	0.14	27
100	8.36	0.36	1.32	1.38	0.43	45	38	34	16	0.34	0.10	30
150	7.64	0.37	1.44	1.46	0.46	46	34	34	16	0.42	0.18	30
200	7.99	0.36	1.38	1.31	0.50	55	42	38	19	0.44	0.10	29
0.2 P.P.M. Atrazine												
0	6.86	0.16	1.34	1.18	0.38	45	44	28	17	0.30	0.12	22
50	6.78	0.16	1.25	1.36	0.44	43	38	32	16	0.32	0.11	23
100	7.35	0.30	1.52	1.39	0.46	47	42	34	17	0.42	0.10	30
150	6.81	0.36	1.51	1.40	0.50	42	38	36	17	0.42	0.11	30
200	9.08	0.40	1.20	1.25	0.44	40	39	30	15	0.41	0.10	27
0.4 P.P.M. Atrazine												
0	6.20	0.16	1.65	1.24	0.45	47	40	29	16	0.38	0.11	22
50	8.51	0.31	1.48	1.32	0.49	55	40	39	19	0.52	0.13	30
100	6.60	0.28	1.74	1.36	0.48	54	45	26	20	0.43	0.12	30
150	7.45	0.37	1.56	1.46	0.46	48	32	28	16	0.52	0.12	31
200	6.10	0.41	1.16	1.52	0.44	42	32	25	16	0.52	0.10	32
Webster Clay Loam, No Atrazine												
0	4.93	0.22	0.92	1.12	0.46	74	48	46	30	0.37	0.07	39
50	5.67	0.31	0.98	1.24	0.49	79	54	46	32	0.42	0.07	44
100	5.11	0.38	0.98	1.35	0.53	90	51	45	30	0.44	0.07	46
150	5.02	0.40	0.91	1.14	0.43	65	41	42	28	0.48	0.06	41
200	4.36	0.42	0.79	1.02	0.40	57	42	42	28	0.29	0.06	38
0.2 P.P.M. Atrazine												
0	4.10	0.16	0.88	1.00	0.42	77	58	40	28	0.26	0.06	37
50	5.45	0.32	0.98	1.16	0.48	66	54	47	32	0.38	0.06	42
100	4.80	0.36	1.00	1.09	0.42	72	36	41	29	0.33	0.05	42
150	4.08	0.35	0.96	0.94	0.38	58	40	40	28	0.25	0.05	37
200	5.73	0.44	0.89	1.03	0.41	76	51	45	30	0.34	0.06	40
0.4 P.P.M. Atrazine												
0	4.31	0.22	0.93	0.91	0.42	90	59	38	29	0.32	0.05	36
50	4.74	0.26	0.98	0.99	0.43	78	45	39	30	0.36	0.05	38
100	4.30	0.34	0.90	1.02	0.41	66	43	45	30	0.30	0.06	39
150	4.09	0.39	1.02	1.04	0.44	68	54	50	32	0.38	0.06	38
200	3.63	0.37	0.92	0.92	0.37	62	47	40	28	0.32	0.05	36

Table III. Results of Analysis of Variance for Growth and Mineral Composition of Soybean Shoots Grown on Three Soils with Three Atrazine and Five Phosphorus Treatments

Main and Interaction Effects	Growth	P	K	Ca	Mg	Fe	Mn	B	Zn	Mo	Si	Ba
Concentration of Mineral Ion per Unit Dry Plant Material												
Soils	[a]	[a]	[a]	[a]	[b]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Atrazine	[a]	[a]	[a]	[b]	[c]	[b]	N.S.[d]	N.S.	[a]	[a]	[a]	[a]
Phosphorus	[b]	[a]	[c]	[a]	N.S.	[c]	[b]	N.S.	N.S.	[c]	N.S.	[a]
Atrazine × phosphorus	[a]	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	[b]	N.S.	N.S.	N.S.	N.S.
Soils × atrazine × phosphorus	[b]	[a]	[a]	[b]	N.S.	N.S.	[c]	[b]	[a]	[a]	[a]	[a]
Total Accumulation of Mineral Elements												
Soils	...	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Atrazine	...	[a]	N.S.	[a]	[a]	[a]	[a]	[a]	[a]	[b]	[a]	[a]
Phosphorus	...	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	N.S.	[a]
Atrazine × phosphorus	...	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[b]	[c]	N.S.	[a]
Soils × atrazine × phosphorus	...	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[b]	[b]	[a]

[a] Significant at 1% level.
[b] Significant at 5% level.
[c] Approaching significant (significant at 10% level).
[d] N.S. Not significant.

Figure 1. Effects of atrazine-phosphorus interaction on growth

Pots 1 through 5. Soil contained 0.4 p.p.m. atrazine and phosphorus as $Ca(H_2PO_4).H_2O$ at following rates: 1, none; 2, 50 p.p.m.; 3, 100 p.p.m.; 4, 150 p.p.m.; 5, 200 p.p.m.
Pots 6 through 10. Soil contained no atrazine and phosphorus at following rates: 6, none; 7, 50 p.p.m.; 8, 100 p.p.m.; 9, 150 p.p.m; 10, 200 p.p.m.

loam samples. However, the trend was present with the latter.

Considering growth and mineral composition of the soybean shoots, the wide and highly significant differences between soils is readily apparent. Growth and the content of potassium, phosphorus, calcium, silicon, manganese, boron, zinc, aluminum, molybdenum, sodium, and barium in the soybean shoots varied significantly with soil type. If evaluated on the basis of total accumulation of the mineral element, magnesium, iron, and strontium could be added to the list.

When evaluating data referring to the mineral composition of plants one must not fail to consider that growth is intimately associated with the results obtained. If total mineral uptake is unaffected by a treatment that stimulates or inhibits growth, the concentration of the element in the plant is, respectively, diluted or concentrated. On the other hand, the mineral concentration in the tissue may be unaffected, but growth and total accumulation may vary. The data obtained with the Forman clay loam sample in this study illustrated what happens when total uptake of a given element did not decrease as much as growth. Nearly all elements increased in concentration in soybean shoots with the addition of 0.4 p.p.m. of atrazine to the soil.

Elements considered mobile in a plant are sometimes reported in the literature on the basis of total accumulation and immobile elements on the basis of concentration in the plant tissue. Ohlrogge (1960) reviewed the mineral nutrition of soybeans and classified mineral nutrients according to their mobility in the soybean plant. In the study reported here, no clear relationship between the method of quantitating mineral content and those elements classified by Ohlrogge as mobile or immobile could be detected. Consequently, expressing the data in terms of concentration seemed most advantageous. Total accumulation may be determined by multiplying growth by the concentration of the mineral element in the plant tops.

Fletcher (1961) found, at the five-leaf stage, that addition of large amounts of phosphorus as superphosphate increased the leaf content of phosphorus, manganese, calcium, boron, potassium, silicon, and sodium. The accumulation of copper and magnesium was depressed and iron unaffected. Rates in his study were as high as 2240 p.p.m. of phosphorus. In the present study, the application of phosphorus fertilizer significantly increased the shoot content of phosphorus, molybdenum, calcium, and barium. The manganese content of the shoots was significantly depressed and zinc content tended to be depressed. Atrazine significantly affected the shoot content of calcium, silicon, boron, strontium, and molybdenum in two of the three soil samples studied. In terms of concentration of a given element in the shoot tissue, few significant effects were observed due to a phosphorus atrazine interaction. However, of elements reported in Table II, only silicon and molybdenum (total accumulation) were unaffected by this interaction on at least two of the three soils. Since growth was affected by the interaction, interpretation of the latter results is difficult. The authors feel that only where differences in both the concentration of the element in the shoot tissue and the total accumulation of the element by the shoot are significant can one reliably claim an interaction. Setting these requirements, an atrazine-phosphorus interaction was shown with the accumulation of magnesium, manganese, boron, and barium by soybeans grown on Forman clay loam.

If, as suggested by several workers (Bear, 1950; Mehlich and Reed, 1948; Wallace *et al.*, 1948), the total accumulation of cations or anions remains constant, any increase in accumulation of one ion must be balanced by a decrease in one or more ions. Data in Table IV show that, in general, with any given atrazine treatment the sum of the accumulation of potassium, calcium, and magnesium remained constant. Sodium was ignored because its accumulation was too small to alter the calculation. No interaction or effect of phosphorus was observed. However, each increment of atrazine increased the total accumulation of these cations by the soybean shoots grown on Forman and Buse clay loam samples; in shoots grown on the Webster clay loam sample they tended to decrease.

DeKock and Hall (1962) found that calcium accumulation decreased as potassium accumulation increased. Striking effects on the Ca/K ratio were observed in this study. A significant atrazine-phosphorus interaction was found in shoots grown on both the Forman and Buse clay loam samples. With the Forman clay loam treatments containing the highest rate of atrazine the ratio increased as applied phosphorus increased. Curiously, the opposite effect was observed with the Buse clay loam. These two soil series are found in the same soil association. These data point out the value of ratios in establishing significant effects where two ions are affected differently but not necessarily significantly.

Phosphorus fertilization has frequently been reported to depress the uptake of iron by soybean plants. The data reported here do not clearly demonstrate this response. However, the manganese concentration was affected by both atrazine and phosphorus and a significant interaction between these two chemicals was observed with the Forman clay loam. Excessively high or low Fe/Mn ratios are considered by some as toxic to plants. In this study, phosphorus, but not atrazine, was shown to affect these ratios, with some suggestion of an interaction. The effect on manganese accumulation and consequently Fe/Mn ratios by different rates of phosphorus in this and other studies conducted at this laboratory have been typically nonlinear. Somers *et al.* (1942) considered Fe/Mn ratios below 1.5 or above 2.5 unfavorable to plants. On that basis, Fe/Mn ratios in soybeans grown on the Forman and Buse clay loam samples were approaching a level where toxicity effects appear. However, variations in Fe/Mn ratios in this study were not consistent with visual symptoms and growth, and it is doubtful that they contributed directly to the observed interaction. Whether manganese is free to perform its metabolic or physiologic function in the plant cannot be determined by total mineral analyses.

The severe injury observed in Figure 1 could result from an increased sensitivity of the soybean to either phosphorus or atrazine. Fletcher (1961) observed a marked increase in manganese accumulation with high rates of phosphorus fertilization. On the other hand, Millikan *et al.* (1966) reported that manganese accumulation was depressed by simazine. Phosphorus depressed manganese uptake by the soybean shoots at the rates used in this study. These results were not necessarily inconsistent with those of Fletcher (1961), who found sharp increases of manganese accumulation only at high rates of phosphorus. Atrazine alone served to increase manganese content of the soybean shoot. These observations were contrary to those of Millikan *et al.* (1966), but again the

Table IV. Sums of Potassium, Calcium, and Magnesium, and Ca/K and Fe/Mn Ratios in Soybean Shoots Grown on Three Soils with Three Atrazine Treatments and Five Phosphorus Treatments

$Ca(H_2PO_4)$ Applied, P.P.M.	K + Ca + Mg, Meq./100 G.			Ca/K, Meq./Meq.			Fe/Mn, P.P.M./P.P.M.		
	Atrazine, P.P.M.								
	0	0.2	0.4	0	0.2	0.4	0	0.2	0.4
	Borman Clay Loam								
0	134	124	190	0.44	0.44	0.57	1.04	1.10	1.02
50	150	131	198	0.32	0.43	0.57	1.00	1.39	1.08
100	151	166	205	0.33	0.40	0.68	1.10	1.07	1.46
150	137	172	208	0.34	0.48	0.70	1.12	0.96	1.44
200	149	168	203	0.32	0.39	0.70	0.89	0.97	1.11
	Buse Clay Loam								
0	131	124	141	0.53	0.57	0.66	1.20	1.03	1.17
50	151	132	143	0.47	0.49	0.56	1.15	1.14	1.37
100	138	145	150	0.47	0.55	0.61	1.19	1.10	1.17
150	147	148	151	0.49	0.55	0.53	1.31	1.10	1.50
200	110	129	142	0.52	0.47	0.38	1.29	1.02	1.27
	Webster Clay Loam								
0	116	107	101	0.41	0.43	0.52	1.53	1.32	1.50
50	127	123	106	0.39	0.42	0.50	1.44	1.20	1.75
100	135	114	107	0.36	0.46	0.44	1.77	1.92	1.54
150	114	102	110	0.40	0.51	0.52	1.58	1.45	1.24
200	102	108	100	0.38	0.43	0.49	1.33	1.49	1.30

latter workers used higher rates of herbicide than used in this study. The phosphorus-atrazine treatments reduced the accumulation of manganese by soybeans. These results were more consistent with those reported by Millikan *et al.* In addition, necrosis typical of atrazine injury appeared in leaves of soybeans growing in the phosphorus-atrazine treatments. From these observations phosphorus appeared to have increased the sensitivity of soybeans to atrazine. Further work is necessary to establish the mechanism of this interaction.

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