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VEGETABLE CROPS IN RELATION TO SOIL FERTILITY: II. VITAMIN C AND NITROGEN FERTILIZERS

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The effects of soil fertility on the nutrient or dietary value of vegetable crops deserve increasing attention in experimental studies. Many investigators are agreed that vegetables are nutritionally superior when produced on soils well supplied with the essential fertility elements, and nutritionally inferior when grown on infertile soils.

With increased plant yields due to the applications of fertilizers, there is usually associated an increase in the total content and concentration of minerals in the plants, but not so frequently of vitamins. If the functions of vitamins in the plant are those of catalytic agents, as described by Schopfer and others (32, pp. 194–197), one would not necessarily expect the concentration of these catalysts to be greater, merely because of an increase in plant size. Rather, a rise in the concentration of the catalyst might indicate a natural plant mechanism to make more effective some element that is deficient in the soil, much as the thyroid enlarges in the human body in an attempt to produce more thyroxine when iodine is deficient, and as parathyroid glands increase their hormone production under calcium deficiencies (6).

This report, which confirms the above hypothesis, gives evidence of an inverse relationship between the concentration of vitamin C in plant tissue and nitrogen supplied as fertilizer, together with the usually positive correlation between this soil treatment and the yields of spinach and Swiss chard. In short, the experiments indicate that the concentration of vitamin C in these leafy green vegetables increases as the fertility of the soil with respect to nitrogen decreases.[2]

HISTORICAL

Numerous studies have been reported wherein applications of different fertilizers caused increases in the concentration of vitamin C (13, 14, 15, 16, 27, 28), whereas other reports concluded that they do not bring about such increases (19, 23, 31, 34). And now considerable experimental work indicates that the application of fertilizers, particularly nitrogenous ones, may reduce rather than increase the concentration of vitamin C in many vegetable and fruit crops.

Some of the earliest work performed was that of Bracewell, Hoyle, Wallace, and Zilva (4, 5, 35), on the antiscorbutic potency of apples. They concluded that the vitamin was in greater concentration in apples of low nitrogen supply. With all varieties tested, under

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[2]In this study, concentrations of vitamin C in the leaves and stems and not in the fruit or seed are considered, because vegetative parts portray a truer index of the influence of variable soil fertility than do reproductive organs, which are necessarily more constant in their chemical composition.

the various conditions of nitrogen fertilization, an inverse relationship existed between the nitrogen content of the fruit and the concentration of vitamin C. Similar conclusions were reached by Kessler (20), who reported that fertilizing apple trees with nitrogen depressed the ascorbic acid content of the fruit progressively as the amount of fertilizer was increased. Hahn and Görbing (9) obtained comparable results with spinach.

In several investigations on tomatoes the influence of fertilizers on vitamin content could be noted. Ott (26) reported that tomatoes grown without fertilizer contained more vitamin C than those fertilized with nitrogen, phosphorus, and potassium. Similarly, Maclinn and Fellers (24) found that tomatoes grown under a low nitrogen supply contained 96–136 units of vitamin C, whereas those under high nitrogen had 74–114 units, but the effects of light and nitrogen were not separated in this experiment. More recently very extensive experiments on the tomato have been conducted by Lyon and his co-workers (11, 22). In their nutritional studies, plants grown with sulfate and nitrogen deficiencies resulted in fruit of a higher vitamin C content. The lack of iron produced a significant rise in ascorbic acid.

Again verifying this inverse relationship, Hamdallah (10) found that the vitamin C content of plants which were grown without iron or magnesium was as great as or greater than that of plants fed normally, even though the former were smaller and chlorotic in appearance. In a study of Sudan grass, Wynd (36) reported that the total yield of vitamin C per acre varied inversely as the yield of dry matter and the delivery of nitrogen by the crop. Reder, Ascham, and Eheart (29) studied the effects of fertilizers and environment on the ascorbic acid content of turnip greens and found that potassium and nitrogen produced significant decreases in ascorbic acid. A recent report on grapefruit by workers (18) in Arizona demonstrated an inverse relationship between vitamin C in the juice and nitrogen fertilization.

A careful examination of the literature reveals that environmental factors other than soil nutrient deficiencies may influence the ascorbic acid content. Climatic or other environmental conditions unfavorable for growth frequently result in increased concentrations of vitamin C. Sunny and dry weather, which may reduce the rate of vegetative development, is more conducive to the accumulation of vitamin C than are cloudy and rainy periods (29). Strong light under some conditions has long been recognized as an inhibitor of plant growth, yet light is very influential in increasing the vitamin C content in the plant (12, 30). It is reported (2, 21) that crops grown at high altitudes have an ascorbic acid content sometimes two to three times that of those at lower elevations. Here, again, the high vitamin content is associated with stunted plants grown in an environment unfavorable for maximum vegetative development.

Ascorbic acid is of universal occurrence in growing plants, and it has been demonstrated that there is an enormous increase in its concentration within the germinating seed (25, 30). Bonner and Bonner (3) and Dennison (7) have suggested that it is an essential factor in plant growth. Accordingly, its presence in the plant has been considered something other than merely an accumulation of one of the by-products of metabolism, since it has also been suggested that vitamin C serves as a catalyst in respiration (17, 33). As such, it is allied closely to some of the mineral elements (Fe, Cu, Mn); both are similarly concerned with oxidative enzyme systems. This relationship has been demonstrated, in part, by Elliott and Libet (8), who have shown that an ascorbic acid-iron system exists in the oxidation of phospholipids. Ascorbic acid and a very small amount of iron when used together caused great stimulation of respiration; either one used alone produced some acceleration.

PROCEDURE AND METHODS

Spinach, the Bloomsdale Long Standing variety, was grown under twenty levels of soil fertility in the greenhouse during the winter of 1941–42. Variable amounts of exchangeable ions placed on the clay subsoil of Putnam silt loam offered a means of controlling the fertility of the soil. Since nearly half of the exchange capacity of this natural clay is taken by hydrogen, various nutrients adsorbed on the clay in exchangeable form may be provided for plants in any desired ratios and quantities, by replacing, more or less completely, the hydrogen by the selected cations and by using the proper amount of the prepared clay in its admixture with sand. The stability of the clay and its naturally high hydrogen content make its use, by simple additions of cations as exchanges for its hydrogen, very convenient.

A series of clay aliquots was prepared by adding calcium acetate to provide 0, 5, 10, 20, and 40 m.e. of calcium. Then from four aliquots of each of these levels of calcium was prepared a nitrogen series by adding 5, 10, 20, and 40 m.e. of nitrogen as ammonium nitrate. This provided, then, twenty different soil treatments giving four levels of nitrogen, each of which had five variable amounts of calcium combined with it as additions to the supply native in the initial clay. To each of these individual treatments were added other nutrients in constant quantities. These additions consisted of 20 m.e. each of potassium and phosphorous and 6 m.e. each of magnesium and sulfate.

The quantity of subsoil clay required to provide the exact exchange capacity for the added nutrients in each treatment was determined beforehand in terms of the known qualities of the clay. This Putnam subsoil material was then mixed under moisture with the particular nutrients and homogeneously blended with pure white quartz sand. Ten replicates of each treatment, or a total of 200 of the mixtures of sand and clay, each in a 1-gallon glazed crock, were prepared. One plant was grown in each. The treatments were randomized on benches and the crocks spaced sufficiently to eliminate light variations. Five of the replicates served for determination of the concentration of vitamin C, while the other five were used for mineral analyses. Entire plants with the exception of roots were utilized.

In the vitamin C determinations, sampling was adjusted such that one plant from each of the twenty treatments was collected at a time, the analyses being completed within 2 to 3 hours after dismantling. This procedure was repeated on consecutive days until the five plants in each treatment were analyzed. In preparation for analyses the material was ground homogeneously in a Waring blendor, and vitamin C was determined by modifying slightly the dye reduction method of Bessey and King (1). The vitamin concentration was expressed in milligrams of ascorbic acid per 100 gm. of fresh material.

For the mineral analyses, the plants were collected along with those taken for determination of vitamin C and prepared according to usual methods of harvesting, washing, drying, and preservation. Both fresh and dry weights were recorded. Some determinations were made spectrographically, and others were by regular methods recognized as standard laboratory procedures.

RESULTS

The outstanding features of the crop behavior as influenced by the different fertility levels of the soil were the negative correlation of the concentration of vitamin C with the yields (fresh weights) of the crop; the yields of the spinach as a more direct function of the amount of applied nitrogen; the more decided response by the crop to the different levels of nitrogen than to those of calcium;

TABLE 1

*Crop yield, and concentration of ascorbic acid and nutrient elements of spinach under variable levels of calcium and nitrogen offered in the soil**

TREATMENT NUMBER	APPLIED N	APPLIED Ca	ASCORBIC ACID/100 GM.†	YIELD /10 PLANTS	MINERAL ANALYSES OF PLANTS N	Ca	P	K	Mg	Mn
	m.e.	*m.e.*	*mgm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	40	40	70.5 ± 4.7	234.35	6.20	0.71	0.64	8.19	1.25	0.018
5	40	20	84.2 ± 3.6	320.49	6.30	0.69	0.77	7.47	1.03	0.025
9	40	10	88.6 ± 8.5	179.05	6.20	0.64	0.52	7.63	1.05	0.038
13	40	5	82.4 ± 4.8	128.55	6.20	0.79	0.61	7.80	1.07	0.025
17	40	0	120.6 ±11.1	67.75	6.15	0.63	0.72	7.41	1.06	0.028
Average.......			89.3 ± 5.0	186.04	6.21	0.69	0.65	7.70	1.09	0.027
2	20	40	95.1 ± 2.8	170.80	4.90	0.80	0.82	8.29	0.99	0.020
6	20	20	108.1 ± 8.1	220.05	5.20	0.64	1.06	8.02	0.85	0.034
10	20	10	87.8 ± 8.1	229.00	5.20	0.64	0.76	6.86	0.91	0.031
14	20	5	81.2 ± 7.0	205.10	5.40	0.59	0.72	7.49	0.83	0.034
18	20	0	124.5 ± 3.8	197.32	5.40	0.50	0.77	7.88	0.92	0.033
Average.......			99.4 ± 4.6	204.45	5.22	0.63	0.83	7.71	0.90	0.030
3	10	40	170.2 ±13.6	85.03	3.20	1.13	1.91	7.92	0.68	0.032
7	10	20	134.1 ±10.1	108.45	3.10	0.75	1.41	8.39	0.70	0.034
11	10	10	113.4 ±11.7	138.50	3.75	0.66	1.05	7.39	0.83	0.046
15	10	5	184.4 ± 8.5	153.09	4.95	0.68	0.88	8.07	0.89	0.033
19	10	0	126.5 ±12.9	99.90	3.85	0.45	1.01	6.92	0.53	0.030
Average.......			145.7 ± 8.1‡	116.99	3.77	0.73	1.25	7.74	0.73	0.035
4	5	40	159.9 ± 8.2	36.49	4.05	1.23	2.61	8.31	0.15	0.016
8	5	20	181.9 ±18.7	65.37	3.35	0.86	1.96	8.68	0.87	0.022
12	5	10	144.0 ±19.5	114.90	4.60	0.86	1.08	8.23	0.93	0.034
16	5	5	145.6 ±10.5	60.10	3.15	0.62	1.31	6.71	0.54	0.049
20	5	0	178.4 ±11.0	76.70	4.20	0.59	1.26	6.86	0.62	0.040
Average.......			162.0 ± 7.6‡	70.71	3.87	0.83	1.64	7.76	0.62	0.032

* Ascorbic acid per 100 gm. fresh weight, yield in fresh weight per 10 plants, and mineral analyses in percentage of dry weight.

† Each value represents mean concentration with its standard error of five plants or replicates in each treatment.

‡ Mean concentration is greater beyond 1 per cent level of significance than means of treatments receiving 40 or 20 m.e. of nitrogen.

and the relations in concentrations of calcium and phosphorus with that of the vitamin C.

These facts are evident from the summary of the results with spinach as presented in table 1, wherein the treatments for spinach are arranged in groups of different calcium levels with the same quantity of nitrogen, and then the values averaged for each nitrogen level. Included with the data for concentrations of ascorbic acid and yields of plants are the analyses for six soil fertility elements, viz., nitrogen, calcium, phosphorus, potassium, magnesium, and manganese. It is possible to rearrange these data into groups of four different nitrogen levels, averaged for each constant calcium group, to show the influences of the variable calcium. By this means, its effect regardless of nitrogen can be measured.

That the vitamin concentration in the spinach was increased as the nitrogen supplied in the soil was decreased is evident from the table. The yields suggest the characteristic sigmoid curve of decided increase at certain increments in the levels of offered nitrogen, and then no further increase—possibly decrease—at the higher levels of this applied nutrient. The corresponding yields expressed on a dry weight basis portrayed the same correlations with nitrogen additions and vitamin C concentrations as did the yields in terms of fresh weights, and accordingly are not given.

TABLE 2

Ascorbic acid and calcium in spinach crop as related to yield and nitrogen supplied

Nitrogen applied............... *m.e.*	40	20	10	5
Yield/10 plants................. *gm.*	186.0	204.5	117.0	70.7
Ascorbic acid/10 plants........ *mgm.*	166.1	203.2	170.5	114.6
Calcium/10 plants............. *mgm.*	1412.0	1416.8	939.4	645.6

Striking correlations of vitamin C with the mineral composition are evident. When the concentrations of ascorbic acid were high in spinach, those of nitrogen and magnesium were low. Neither potassium nor manganese showed any connection with vitamin C. Of added interest is the fact that the concentrations of calcium and phosphorus—usually associated more prominently with anabolic than with respiratory processes—are parallel with those of ascorbic acid.

Some thought may well be given to the fact that the supplies of calcium and phosphorus were constant for each decreasing nitrogen level in the soil, yet these two nutrients moved into the crop in amounts that ran almost parallel with the quantities of vitamin C synthesized. Also of interest is the fact that the total vitamin C accumulation in the crop was less at 40 than at 10 m.e. of applied nitrogen, yet yields were increased by 59 per cent at the higher nutrient level (table 2). Total accumulations of ascorbic acid and calcium suggest that as deficiencies in the soil fertility (nitrogen) produced lower yields of crops, the plants were richer not only in their concentration of this vitamin, but also in calcium and phosphorus. The percentages of total nitrogen, of course, were decidedly lower.

This particular chemical behavior seems reasonable in view of other works dealing with vitamin C and mineral nutrition. Frequently among those reported, there were indications that the greater concentrations and yields of vitamin C occurred, not when optimum conditions of fertility were at hand, but under mineral deficiencies.

It is conceivable that with limitations in the mineral supply, the vitamin C content might increase, since, as previously pointed out, ascorbic acid and some of the minor elements (Fe, Cu, and Mn) "function as coenzymes or fragments of coenzymes" (32) in cellular oxidations. Might the increase in the vitamin concentration of plants serve to offset, in part, deficiencies of essential mineral nutrients, and conversely, an optimum mineral supply in the soil depress the vitamin concentration? Plants may be equipped with more than one mechanism of accomplishing a given reaction or completing an essential process for which, if one means fails or becomes limiting, a substitute may be called in.

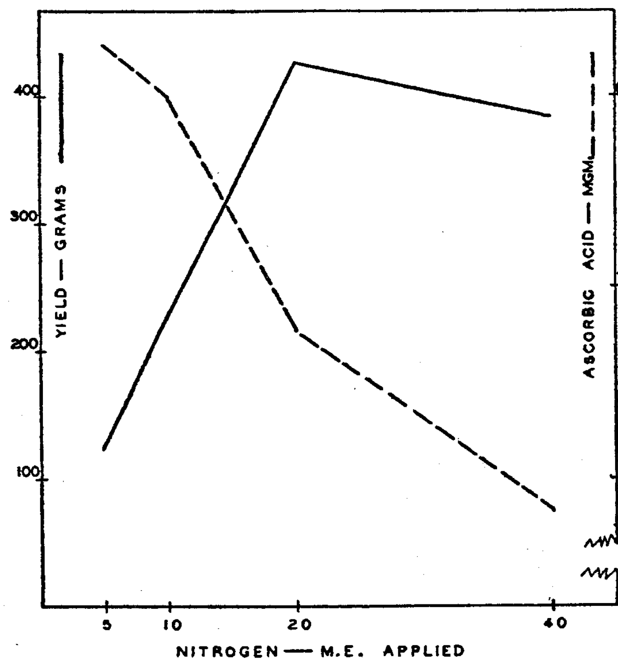

FIG. 1. CONCENTRATIONS OF ASCORBIC ACID AND VEGETATIVE YIELDS OF SWISS CHARD AS RELATED TO NITROGEN ADDITIONS TO THE SOIL

As a confirmation of the results obtained for spinach, Swiss chard was grown during the winter of 1943–44. The experimental conditions were identical to those previously used for spinach. Similar clay subsoil and ratios of calcium and nitrogen were utilized. The summarized results of the influence of different levels of soil nitrogen on the yield of the crop and its ascorbic acid composition, as given in figure 1, portray a repetition of the data already presented for spinach. Again, it was found that with a depression of yields due to deficiencies in soil minerals, the percentage of ascorbic acid in the crop increased; and, conversely, with increased growth and production of vegetative bulk, the concentration of vitamin decreased.

SUMMARY AND CONCLUSIONS

A careful review of the literature indicates that a high rather than a low vitamin C concentration in plants is associated with a reduction in yield due to nutrient deficiencies, particularly nitrogen. Since certain minerals and ascorbic acid play similar roles as catalysts in plant metabolism, it is suggested that the increase in vitamin C may be a secondary mechanism of the plant to overcome unfavorable conditions of mineral nutrition.

Evidence is presented which indicates that the concentration of vitamin C in leafy green vegetables increases as the fertility of the soil with respect to nitrogen decreases. The following facts were established by the investigation:

There was a decrease in the concentration of ascorbic acid with increasing quantities of nitrogen applied as fertilizer.

A negative correlation was indicated between the yields of the crop and its concentration of ascorbic acid.

There was a decided crop response to nitrogen—as reflected by yields, vitamin C concentration, and mineral content—in some instances quite independent of the calcium levels with which the nitrogen was combined.

The concentrations of calcium and phosphorus within the plant suggest their relation with those for vitamin C.

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