THE INFLUENCE OF MINERAL NUTRITION, SOIL FERTILITY, AND CLIMATE ON CAROTENE AND ASCORBIC ACID CONTENT OF TURNIP GREENS

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(WITH FOUR FIGURES)

Previous investigations from this laboratory with tomatoes (**2, 7**) have indicated that the ascorbic acid and carotene content of the tomato fruit is influenced but little by the supply of mineral nutrients to the plants. This work was done with a fruit, however, and it was thought that in a leafy crop like turnip greens the influence of mineral nutrient supply and soil fertility upon these two constituents might be greater than in the tomato. The two experiments reported here are concerned with the effect of the supply of nutrient elements in sand and in soil cultures on the vitamin content of turnip greens. Subsequent experiments to be reported elsewhere will deal with the effects of other environmental factors. In all experiments the Shogoin variety of turnips was used.

Several investigators (**11, 14, 16**) have noted that the content of several vitamins and minerals is relatively high in turnip greens. This particular investigation deals with the ascorbic acid and carotene contents.

KELLER and MINOT (**9**) report that the ascorbic acid content of fresh turnip greens varied from 75–160 mg. per 100 gm. fresh weight (with an average of 115 mg.) in ten samples. TUL'SCHINSKAYA (**18**) found in each 100 grams of fresh leaves 51.9 mg. of ascorbic acid in July, 76.7 mg. in August, and 105 mg. in September. FLOYD and FRAPS (**5**) state that the greens vary from 67 to 422 mg. of ascorbic acid per 100 gm. fresh weight. REDER, ASCHAM, and EHEART (**15**) found variations from 116 to 257 mg. per 100 gm. fresh weight. They grew turnips in well-replicated fertilizer experiments at four locations in the south. In their summary they state: "Wide variations were obtained in the ascorbic acid content of greens produced at the four places; the mean ascorbic acid content of greens at Norfolk, Virginia (2.4103 mg. per gm.), was nearly twice that of greens at Blacksburg (1.2842 mg. per gm.). In the four experiments, the influence of place was 13.75 times as great as the most important average effect produced by fertilizer treatment within a single locality. These variations did not appear to be directly related to differences in soil composition or to differences in temperature. . . . These results seem to indicate that the formation of ascorbic acid may be influenced by light intensity and rainfall as well as by fertilizer applications."

Methods of analysis

ASCORBIC ACID

The method of preparing the extracts has been described by MORELL (**13**). The extract of the sample after grinding was filtered through Whatman

no. 2 paper and an aliquot of the clear, colorless filtrate titrated to an end-point with standardized 2,6-dichlorophenol-indophenol dye. Each liter of the acid used for the extraction contained 20 gm. of metaphosphoric acid and 20 ml. of concentrated sulfuric acid. The titration method was checked by comparison with the method of BESSEY (**1**) and found to agree.

DEHYDROASCORBIC ACID

The method employed was a slight modification of the method of GUNSALUS and HAND (**6**). An extract was prepared from 10–25 gm. of fresh turnip leaves in a Waring Blendor with 100 ml. of a 1 to 10 dilution of the acid mixture used for extraction of ascorbic acid; i.e., a mixture of 2 gm. of metaphosphoric acid and 2 ml. of H_2SO_4 per liter. The ground sample was filtered through Whatman no. 2 paper and aliquots titrated for ascorbic acid by the usual method. Additional aliquots were treated as follows: a mixture containing 220 ml. of neutral potassium phosphate buffer (M/15), 20 ml. of 10 per cent. glucose and 20 ml. of bacterial suspension[1] was incubated in a water bath at 40° C. for approximately 15 minutes. To a 25-ml. aliquot of this mixture in a test tube were added 2 ml. of the turnip leaf extracts. The contents of the tube were rapidly mixed and two 5-ml. portions removed immediately for ascorbic acid titration by the usual method. At the end of a 15-minute incubation at 40° C., two additional 5-ml. samples were taken and titrated. The increase in reducing power of the cultures after 15 minutes incubation was ascribed to the conversion of dehydroascorbic acid to ascorbic acid by the bacteria.

CAROTENE

A detailed study of methods of carotene analysis of turnip greens was made, the results of which are to be presented in a separate paper (**3**). The essentials of the method finally selected are as follows: The leaves were killed by immersion in boiling water for 1/2 minute, and the pigments were extracted from the leaves by the method of MOORE and ELY (**12**). After filtration, water was added in the petroleum ether and ethyl alcohol extraction mixture, causing a separation of an alcoholic phase and a petroleum ether phase. The latter layer was removed, and the alcoholic phase was washed once with petroleum ether; two petroleum ether extracts were combined and washed twice with about 200 ml. of tap water. The petroleum ether extract, containing all the carotene and most of the chlorophylls and xanthophylls, was then dried over anhydrous sodium sulfate and made up to a given volume. Aliquots were then run through a starch-super-cell[2] column (2 parts of starch; 1 part super-cell by weight) which retained the chlorophylls and xanthophylls. The petroleum ether containing the carotenoid pigments was collected after passing through the column, made

[1] Prepared according to GUNSALUS and HAND (**6**). PROF. GUNSALUS kindly furnished the stock culture of *Bacterium coli*.

[2] Soluble starch, Indicator grade, from Eimer and Amend.

to an arbitrary volume, and an estimate of the carotene content obtained by use of a colorimeter standardized with β carotene. The method thus determines a group of carotenoid pigments consisting mostly of beta carotene.

Preliminary analyses and sampling techniques for ascorbic acid determinations

Before the turnips from the various experiments were analyzed, some preliminary work on methods of sampling was completed. Consideration was given to the possibility of harvesting the entire top, extracting for either carotene or ascorbic acid, and calculating the vitamin content on the fresh weight basis. This was undesirable since the extraction of the entire top of each individual plant would have involved the use of large quantities of reagents and because harvesting the entire top of plants in certain of the treatments would have involved the inclusion of some dead or partially dead leaves. Therefore, an investigation was made to determine what variation in vitamin content occurred between the leaves of various ages on a given plant and between the different parts of a given leaf in order to determine whether or not one leaf or leaf part might prove representative of the plant as a whole.

In all of this preliminary work, leaves of the Shogoin variety were used. Some of the plants were grown in soil in the greenhouse during the spring for the specific purpose of supplying this material. In part of the preliminary work, leaves were from plants grown in sand culture on a balanced nutrient solution (see treatment 13, figure 1) used in Experiment I.

On May 15, seventeen young leaves (approximately one to two inches in length) were selected from seventeen plants, six weeks old, grown in soil in the greenhouse. Each leaf was divided into basal, medial, and distal fractions of nearly equal area. The corresponding portions of the leaves of all plants were combined to give three samples which were analyzed for ascorbic acid. Similarly, eight older leaves were selected from the middle portion of some of the plants, and these were divided into three samples and analyzed for ascorbic acid as described above. Three of the oldest leaves

TABLE I

The effect of leaf age on ascorbic acid concentration (mg./100 gm. fresh weight) and distribution within the leaf*

Portion of leaf	Youngest leaves (17 leaves used)	Medium-aged leaves (8 leaves used)	Oldest leaves (3 leaves used)
	mg.	*mg.*	*mg.*
Basal end	144.5	107.7	88.9
Middle portion	149.2	115.1	109.1
Distal end	159.5	130.4	113.2
Average entire leaf	151.1	117.7	103.7

* Plants grown six weeks in soil in greenhouse in early spring.

which were of a uniform green color and showed no evidences of necrosis were also selected and treated as above. The results of the analyses are given in table I. There was, in general, an increase in ascorbic acid content from the basal to the distal end of the leaves, and from the oldest to the youngest leaf.

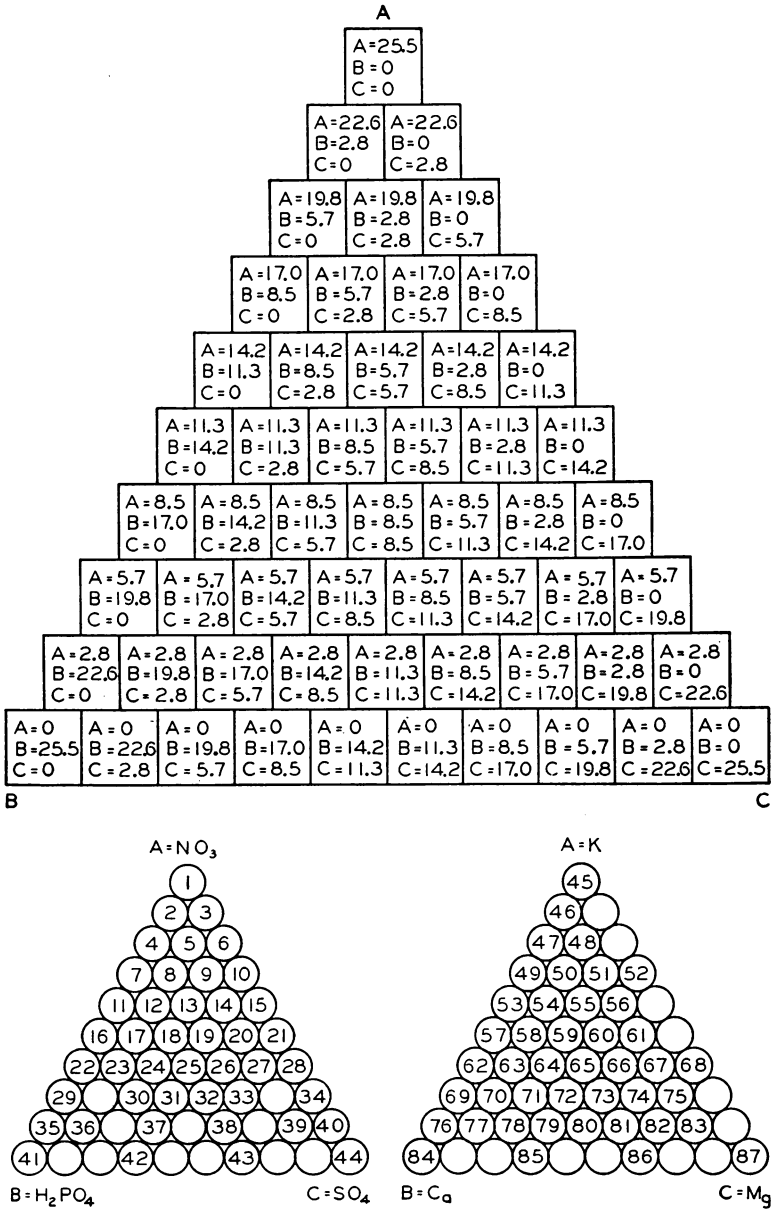

FIG. 1. Upper triangle represents relative proportions of macro-nutrient elements in milliequivalents per liter for 55 possible treatments. Left, Below: 44 combinations of varying anion concentrations. Right, below: 43 combinations of varying cation concentrations. Combinations indicated by treatment numbers.

Additional leaves from the middle portion of other plants in the greenhouse were harvested and each leaf divided longitudinally into two halves by the removal of the midrib which was discarded. Each half of each leaf was separately analyzed for ascorbic acid. It was found that, while there was considerable variation in ascorbic acid content between different leaves, the two halves of any individual leaf were in close agreement with each other.

One week before harvesting the plants in Experiment I for vitamin analyses, additional preliminary work was carried out on comparable plants grown in sand culture and supplied with the balanced nutrient solution used in treatment 13, figure 1. Five plants were selected, and the ascorbic acid content of each leaf from each of the five plants was determined separately. For the sake of simplicity in presenting the results, the average values for the three oldest and the three youngest leaves were calculated; an average value for the remaining middle leaves was also calculated. The results obtained with each plant are presented in table II. As previously found,

TABLE II

THE EFFECT OF LEAF AGE ON ASCORBIC ACID CONCENTRATION (MG./100 GM. FRESH WEIGHT). FIVE PLANTS HARVESTED FROM SAND CULTURE BALANCED NUTRIENT OF FIRST EXPERIMENT

POSITION OF LEAVES	NUMBER OF LEAVES	PLANT NUMBER					AVERAGE
		1	2	3	4	5	
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	
Oldest	3	191.4	166.0	182.4	222.9	201.5	192.8
Middle	2–9	223.2	199.0	210.7	199.7	190.0	204.3
Youngest	3	265.9	220.6	236.3	218.1	198.4	227.9
Average entire plant	.....	219.6	188.8	204.7	209.6	198.9	204.3

there was in general an increase in the ascorbic acid from the oldest to the youngest leaves. The average ascorbic acid content obtained for the five plants by averaging the values obtained for the leaves of various ages is almost exactly the same as the averages of the values obtained for the middle leaves. It thus seems apparent that the middle leaves on the plants may be used as a basis for determination of the ascorbic acid content of the plants.

To check further as to whether or not a representative sample could be obtained by selecting leaves from the middle portion of the plant, another preliminary analysis was made from plants grown in sand culture on the balanced nutrient solution from Experiment I (treatment 13). Instead of selecting the youngest leaves and the oldest leaves on the plant, the leaves from the middle portion were divided into three groups, according to age. The youngest group of these were leaves which were more than four inches in length and thus nearly expanded, and the oldest leaves of this group were all of a uniform green color. The remaining leaves, those of the middle-aged group, represented leaves of an age comparable to those used in all the subsequent analyses reported in the several experiments. The results of the analyses of these leaves were as follows:

Average ascorbic acid content of 20 young leaves 202.0 ± 7.94 mg./100 gm.
Average ascorbic acid content of 40 medium-aged leaves 186.7 ± 4.59 mg./100 gm.
Average ascorbic acid content of 20 older leaves 194.0 ± 6.03 mg./100 gm.

No significant differences in ascorbic acid content were found among the samples of these medium-sized, medium-aged leaves of the plant. The agreement between the three groups was within 8 per cent.

At the time these analyses were made, a number of leaves of the middle-aged group were collected at 9 A.M. and each leaf divided into halves longitudinally by the removal of the midrib. The indivdual half leaves were placed in Petri dishes between layers of moist filter paper and set in the laboratory. One-half of each leaf was analyzed at once and the analyses completed by 11 A.M., and the remainder were analyzed at 10 P.M. on the same day. The latter set had been divided into two groups; one stored at room temperature, and the other in the ice box at 40° F. Both of these groups gave the same ascorbic acid values regardless of storage temperatures, and therefore the results are presented together. The results of these analyses were as follows:

Average ascorbic acid content of 40 leaves at 11 A.M. 186.7 ± 4.59 mg./100 gm.
Average ascorbic acid content of 40 leaves at 10 P.M. 190.5 ± 3.12 mg./100 gm.

Essentially the same ascorbic acid content was obtained with samples analyzed immediately after harvesting or later in the day.

In another preliminary experiment, leaves of middle age were harvested at different times during the day from plants grown outside the greenhouse in sand culture (treatment 13 of Experiment I). The results are presented in table III, both on fresh and dry weight basis. The samples collected at 9 A.M. and 12:30 P.M. agree with each other, while those collected at 4:30 P.M. were slightly higher on a fresh weight basis but not on a dry weight basis.

As a result of this preliminary work, the following procedure was used in sampling the plants in the experiments to be described later. At 9 A.M. medium-aged leaves from individual plants of a given experiment were harvested and placed between layers of moist filter paper contained in Petri dishes. As soon as possible thereafter, each leaf was weighed, the midrib

TABLE III

ASCORBIC ACID CONTENT OF LEAVES HARVESTED AT DIFFERENT TIMES DURING THE DAY. PLANTS GROWN IN SAND CULTURE WITH BALANCED NUTRIENT SOLUTION OF EXPERIMENT I

TIME HARVESTED	NUMBER OF LEAVES	PERCENTAGE DRY WT.	AVERAGE ASCORBIC ACID	
			PER 100 GM. FRESH WT.	PER GM. DRY WT.
		%	*mg.*	*mg.*
9:00 A.M.	10	15.9	205 ± 5.7	129
12:30 P.M.	10	15.3	209 ± 1.7	137
4:30 P.M.	10	16.8	223 ± 5.9	133

excised, and the blade divided longitudinally into halves. Each half was then weighed and again placed in a Petri dish between moist layers of paper. As soon thereafter as possible, one half was analyzed for ascorbic acid, and the other half partially dried in a forced-draft oven at 65° C. and later completely dried in a vacuum oven at 60° C. The percentage of moisture of each leaf was then calculated from fresh and dry weights of the left half of the leaf, and then these dried samples were set aside for subsequent thiamine and nicotinic acid determinations. In most analyses, the ascorbic acid determinations were made within a few hours after sampling; in some, however, as much as six or eight hours elapsed. All analyses were completed on the same day that the leaves were harvested.

Preliminary work on carotene analyses

Preliminary work on carotene analyses was carried out in a manner similar to that described above for ascorbic acid. The results of this work are presented in tables IV to VI. The carotene was distributed fairly uniformly throughout the leaf, and the leaves of different ages had nearly the same carotene concentration (table IV), although the oldest leaves were somewhat lower in carotene than were the other age groups. There was some indication that the carotene content, both on the fresh weight and dry weight basis, decreased during the middle of the day (table VI).

TABLE IV

THE EFFECT OF LEAF AGE ON CAROTENE CONCENTRATION (μ CAROTENE PER GM. FRESH WEIGHT) AND DISTRIBUTION WITHIN THE LEAF*

PORTION OF LEAF	YOUNGEST LEAVES (15 LEAVES USED)	MEDIUM-AGED LEAVES (8 LEAVES USED)	OLDEST LEAVES (5 LEAVES USED)
	μ	μ	μ
Basal end	101.1	107.2	93.9
Middle portion	95.9	100.5	87.7
Distal end	103.2	115.3	85.1
Average entire leaf	99.7	107.3	88.9

* Plants grown six weeks in soil in greenhouse in early spring.

TABLE V

EFFECT OF LEAF AGE ON CAROTENE CONCENTRATION (μ CAROTENE PER GM. FRESH WT.). FIVE PLANTS HARVESTED FROM SAND CULTURES, COMPLETE NUTRIENT OF FIRST EXPERIMENT

POSITION OF LEAVES	NUMBER OF LEAVES	PLANT NUMBER					AVERAGE
		1	2	3	4	5	
		μ	μ	μ	μ	μ	
Oldest	3	34.3	38.5	29.7	41.5	28.3	34.5
Middle	3–5	39.9	34.5	33.0	40.4	38.6	37.3
Youngest	3	40.6	41.0	35.7	38.9	48.4	40.9
Average entire plant	.....	37.6	36.9	34.2	.....	38.1	36.7

TABLE VI

CAROTENE CONTENT OF LEAVES HARVESTED AT DIFFERENT TIMES DURING THE DAY. PLANTS GROWN IN SAND CULTURE, COMPLETE NUTRIENT, EXPERIMENT I

TIME HARVESTED	NUMBER OF LEAVES	PERCENTAGE DRY WT.	AVERAGE CAROTENE, μ/GM.	
			FRESH WT.	DRY WT.
		%	μ	μ
9:00 A.M.	9	14.6	46.5 ± 1.8	318.5
12:30 P.M.	10	16.9	40.2 ± 2.1	237.9
4:30 P.M.	10	17.1	55.8 ± 3.4	326.3

Following the same procedure that was used in the preliminary ascorbic acid work, 72 leaves from the middle portions of the plants were selected and divided into two groups: one consisting of 36 of the younger leaves and the other of 36 of the older leaves. The carotene analyses were as follows:

Average carotene content of 36 younger leaves, 49.5 ± 1.04 mg./gm.
Average carotene content of 36 older-aged leaves, 51.0 ± 0.82 mg./gm.

Thus, within the medium-aged group there was very little difference between the younger and the older leaves.

When leaves were harvested in the morning and one-half analyzed at once, they had essentially the same carotene content as comparable half leaves analyzed later in the afternoon:

Average carotene content of 36 leaves analyzed in A.M., 50.1 ± 0.92 mg./gm.
Average carotene content of 36 leaves analyzed in P.M., 50.4 ± 0.96 mg./gm.

As a result of this work, the same procedure was followed in harvesting samples for carotene analysis as has been described for the ascorbic acid determinations.

Experiment I

By measuring the variations in vitamin content which accompany variations in fertility, an estimate of the relative importance of fertilization on nutritive value may be obtained. Because of the amount of work involved it was arbitrarily decided to limit the work to a study of variations in fertility associated with differences in the supply of the macronutrients (calcium, magnesium, phosphorus, nitrogen, potassium, and sulfur). The variations in fertility of the various treatments was judged by the appearance and yield of the plants produced.

Plants were grown in both sand and soil culture. Through the use of sand culture, it was possible to supply known amounts of mineral nutrients in the various treatments over a wide range. By the selection of soils of low and moderate fertility and through fertilization treatments somewhat analogous to certain of the treatments in the sand cultures, some estimate could be made of the degree to which the results in sand culture, where the supply of mineral nutrients was accurately known, could be interpreted in terms of field practice.

Two soils relatively low in fertility were obtained near Ithaca. Samples of both were brought to the laboratory and placed in crocks comparable to those used in the sand culture work and fertilized in the various ways described below. Field experiments were also conducted at each of the locations where the soils were obtained. At each of these locations pots of each soil were set up and fertilized with one of the balanced fertilizers (treatment 20, Table VII) used in the soil work.

Procedure

The design and procedure used in the sand culture work with turnips was essentially the same as that used in work with tomatoes (**7**). The design was that of a randomized block (**4**) with eighty-seven treatments and four replications. Each replication consisted of a two-pot row, and each pot contained two plants. The replications were randomized by the use of Tippett's randomization tables (**17**). Thus, each treatment consisted of 8 pots with 16 plants. The west plant from each pot was used for the vitamin analyses and the other for growth and dry weight data.

The method of preparing the nutrient solutions has been previously described (**7**). Eighty-seven nutrient solutions were used, and all contained the following concentrations of microelements: 0.5 p.p.m. of B as HBO_3, 0.5 p.p.m. of Mn as $MnCl_2$, 0.05 p.p.m. of Zn as $ZnSO_4$, 0.02 p.p.m. of Cu as $CuSO_4$, and 5.0 p.p.m. of Fe as ferric citrate. In forty-four of the solutions the proportions of nitrate, phosphate, and sulfate were varied, while each contained 12.0 m.e./l. of calcium, 4.5 m.e./l. of potassium, and 9.0 m.e./l. of magnesium. The proportions of potassium, calcium, and magnesium were varied in the remaining forty-three of these solutions, each of which contained 12.0 m.e./l. of nitrate, 4.5 m.e./l. of phosphate, and 9.0 m.e./l. of sulfate (fig. 1). For convenience, the nutrient solutions are represented in cation and anion triangles, and treatment numbers are assigned to those solutions which were used. For purposes of discussion the treatments devoid of one or more ions will be designated as deficient.

For the soil experiments several tons of soil were obtained from the top six inches of each of two fields near the laboratory. One of these soils was a Dunkirk silty clay loam located at Caldwell field; the other was a Mardin silt loam located at Mt. Pleasant Field. Both of these fields were under the supervision of the Cornell University Agronomy Department and, as far as it is known, no differential fertilizer treatments had been applied for the past twenty years. On comparable adjacent fields, plants were known to respond markedly to applications of lime and fertilizers. The soil from both fields was placed in two-gallon crocks, 15 pounds per crock. Five pounds of sand were placed in the bottom of each crock before adding the soil and a glass tube extended from the layer of sand to the surface of the soil in all treatments except one (the puddled soil). Before adding the soil to the crock, the fertilizer and lime (if applied) were mixed thoroughly with the soil of each pot. In those treatments calling for application of manure, alfalfa

TABLE VII

FERTILIZER TREATMENTS APPLIED TO SOIL-POTS AND FIELD PLOTS OF EXPERIMENT I

TREATMENT	LIME PER ACRE	INORGANIC FERTILIZER PER ACRE						APPLICATION TO†				REMARKS
								POT-CULTURES		FIELD PLOTS		
		Ca	K	Mg.	N	P_2O_5	S	DUNKIRK	MARDIN	DUNKIRK	MARDIN	
	tons	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*					
1	.....	.....	.....	.....	.....	.....	.....	+	+	.....	.....	
2	2	.....	.....	.....	.....	.....	.....	+	+	+	+	
3	2	.....	.....	.....	.....	.....	.....	+	.....	.....	.....	Soil puddled
4	10	.....	.....	.....	.....	.....	.....	+	+	+	+	
5	10	.....	.....	.....	.....	.....	.....	.....	.....	+	+	Added microelements*
6	2	.....	.....	.....	.....	.....	.....	.....	.....	+	+	Added microelements*
7	2	29.6	43.6	13.5	20.5	.....	29.7	+	+	+	+	
8	2	29.6	43.6	13.5	.....	.....	59.3	+	+	+	+	
9	2	29.6	43.6	13.5	41.0	.....	.....	+	+	+	+	
10	2	29.6	43.6	13.5	.....	105.0	29.7	+	+	+	+	
11	2	29.6	43.6	13.5	.....	210.0	.....	+	+	+	+	
12	2	29.6	43.6	13.5	20.5	105.0	.....	+	+	+	+	
13	2	37.0	.....	22.5	16.4	63.6	17.8	+	+	+	+	
14	2	.....	.....	45.0	16.4	63.6	17.8	+	+	+	+	
15	2	74.0	.....	.....	16.4	63.6	17.8	+	+	+	+	
16	2	37.0	72.0	.....	16.4	63.6	17.8	+	+	+	+	
17	2	.....	144.0	.....	16.4	63.6	17.8	+	+	+	+	
18	2	.....	72.0	22.5	16.4	63.6	17.8	+	+	+	+	
19	.....	24.7	48.0	15.0	16.4	63.6	17.8	+	+	.....	.....	
20	2	24.7	48.0	15.0	16.4	63.6	17.8	+	+	+	+	
21	2	24.7	48.0	15.0	16.4	63.6	17.8	+	+	+	+	Added microelements*
22	10	24.7	48.0	15.0	16.4	63.6	17.8	+	+	+	+	
23	10	24.7	48.0	15.0	16.4	63.6	17.8	.....	.....	+	+	Added microelements*
24	2	29.6	43.6	13.5	13.6	70.0	19.8	+	+	+	+	
25	2	.....	.....	.....	.....	.....	.....	+	+	.....	.....	Ash of 10 tons of manure
26	2	24.7	48.0	15.0	16.4	63.6	17.8	+	+	.....	.....	Ash of 10 tons of manure per acre
27	2	.....	.....	.....	.....	.....	.....	+	+	+	+	Ten tons of manure per acre
28	2	24.7	48.0	15.0	16.4	63.6	17.8	+	+	+	+	Ten tons of manure per acre

* Twenty-five pounds per acre each of manganese sulfate, copper sulfate, zinc sulfate, and boric acid.
† The symbol + indicates that the fertilizers to the left have been added.

meal, or ash of organic material the application was made by mixing the material with the soil of the middle third of the pot to simulate the position of a plow-down incorporation.

Each treatment with each soil consisted of ten pots with two pots in each of five blocks. In each block all pots were randomized by use of TIPPETT'S randomization tables. Thus, there was a total of 490 pots, with 24 treatments on each of two soils and one additional treatment on one soil. Most treatments were identical for the field plots and the soil pots, but a few of them were applied to the soil pots and not to the field plots and vice versa. Thus, a total of 28 treatments was used and each, regardless of where it was applied, was given a number.

The field areas were 100×62 feet, and these were laid out in 31 rows, extending the length of the field and two feet apart. Alternate rows of plants, with the exception of the outermost two rows, were used as experimental rows. A given treatment was applied to nine feet of a given row, and on any particular row the treatments were separated from one another by a one-foot portion which was not fertilized or harvested. The field was divided into six equal blocks in such a manner that each row extended through two blocks. Each of 25 treatments occurred once in each of the six blocks. The treatments were randomized within a given block by the use of TIPPETT'S randomization tables (**17**). The fertilizers were applied just before planting and worked into the top two inches of the soil for six inches on either side of the row.

Seeds were planted in the field plots on May 1. On June 9 the seedlings were well established, and the plants were thinned so that there remained one plant in every four inches of each row. Seeds from the same lot were planted in the greenhouse on May 1 in sand contained in pots 4×7 inches. The sand was thoroughly moistened with nutrient solution, and, as the seedlings germinated, they were watered about twice a week with complete nutrient solution (see treatment 13, fig. 1). A suffieient number of seeds was planted in each pot to assure germination of five seeds per pot. As the seedlings became established, plants were removed from each pot so as to leave two uniform seedlings in each. On May 27, when the seedlings were 26 days old, they had approximately 4 foliage leaves and the largest leaf on each plant was approximately 3–4 inches long. At this time, the seedlings were transplanted to two-gallon crocks. Seven hundred and twenty of these crocks contained white quartz sand and 490 contained soil to which the various fertilizer mixtures had been applied. Two seedlings were transplanted to each crock and the plants watered with distilled water. The plants in sand were watered the next day with the respective nutrient solutions as previously described; this was repeated twice each week during the early stages of growth and three times each week during later stages when the plants were fairly large. A sufficient amount of nutrient solution was applied at each watering so that an appreciable amount of liquid dripped from the bottom of the pot. The plants in soil pots were watered with dis-

tilled water as needed, which was only occasionally since rain during the season supplied most of the requirements. Subsequent to transplanting, the plants were grown in the greenhouse for a period of twelve days; on June 8 all pots were removed to a plot of ground outside and arranged in the same design as was used in the greenhouse.

On June 25, at which time the first samples for vitamin analysis were taken, the plants in the more favorable treatments were fairly large and had many leaves. The plants in these treatments had reached a stage at which they might be harvested for greens. On June 25, 26, and 27, samples were collected for carotene analysis from the sand cultures, the soil pots, and the fields, respectively; on June 30 ascorbic acid samples were taken from the sand cultures and soil-pots. On July 2, plants in both fields were sampled for ascorbic acid. On July 5, another set of samples for ascorbic acid analysis was taken from the sand cultures. For any given vitamin determination, one leaf was taken from one of the plants in each pot; two leaves from the two pots of a particular treatment in any given block were combined as a single sample. The two leaves were divided longitudinally into halves by the removal of the midrib and weighed. One half was analyzed for vitamin content and the other half used for dry weight determinations. The remaining plants which had not been used for vitamin analyses were harvested according to the following schedule: soil-pots on July 6; sand cultures on July 7; Caldwell field plots on July 8; and Mt. Pleasant field plots on July 9. At the harvest, the plants were pulled up. The tops were separated from the roots and the roots washed thoroughly, removing all fine, fibrous roots and retaining the main tap root. Fresh weight determinations were made on both roots and tops. The tops were then washed by dipping in three successive containers of distilled water to remove dust. The length and diameter of the enlarged portion of the tap root were measured. The tops and roots were then dried for 48 hours in a forced draft oven at approximately 80° C. and weighed.

RESULTS

During germination and early seedling development, growth was more rapid in the greenhouse than in the field. During the middle and latter part of June, however, the plants in the field, in general, grew much more rapidly and at harvest time were considerably larger than the plants in sand culture or pots. The field plants, unlike those in the greenhouse, were never transplanted. The seedlings in the fields were exposed to the natural fluctuations in temperature, while those at the laboratory were subjected to greenhouse conditions. During early May, the field plants were exposed to below freezing temperatures on several nights. At the time of harvest, the stems of many plants at both fields had elongated and some were in flower. In selecting plants for vitamin analysis at the fields, two plants of each treatment of each block were chosen which showed no signs of bolting. The plants in the pots containing soil at each field resembled closely the plants at the laboratory. These were placed in the field at the time the plants at the laboratory were removed from the greenhouse.

Detailed visual observations were made on the plants at successive stages in their development. In addition, counts were made of the number of leaves and measurements taken of the total leaf length of all plants in certain selected treatments. These data were collected on June 11 and 12 and again one week later. At the time of the final harvest, in addition to determination of fresh and dry weights of roots and tops, measurements were made of the length and diameter of the enlarged portion of the tap root of each individual root. Calculations of the percentage of moisture and root-top ratios were also made. These data are too voluminous to be included here. They all serve to establish the fact that the various treatments employed produced great variations in the growth and development of the plants. Only the data on the fresh weights of the tops of the plants are presented. A brief description of the appearance of the plants is included with the discussion of the results. The growth data for the sand cultures are given in figure 2 and for the soil treatments in table VIII. These growth data inadequately demonstrate the differences between treatments, since the type of growth, amount of chlorosis, number of dead leaves, and general appearance were correlated with differences in treatment.

The average value given for the fresh weight of the tops receiving a given treatment is an average of eight plants. The analysis of variance of the growth data for the plants in sand cultures shows that significant differences between treatments existed within both triangles (at the 1 per cent. level). In the anion triangle (fig. 2) very little growth was obtained in those treatments deficient in nitrate and phosphate, and those treatments deficient in sulfate produced much less growth than the more nearly optimum treatments. In the nitrate-deficient treatments the plants were chlorotic and reddish in color. The plants of the sulfate-deficient treatments were chlorotic, especially between the veins, with a red coloration on the under side of the leaves; those of the phosphate-deficient treatments were of a darker green color than plants in the more nearly optimum treatments with some bronze coloration in the older leaves. Increasing amounts of growth were obtained with increasing supplies of nitrate, up to and including those treatments which received 11.3 milliequivalents of nitrate per liter of nutrient. There was an increase in green coloration correlated with this increasing growth. In the cation triangle (fig. 2) little growth was obtained after transplanting in the treatments deficient in potassium and calcium; growth was significantly less than the optimum in treatments deficient in magnesium. All plants in the treatments deficient in magnesium and potassium were distinctly chlorotic. The chlorosis was particularly apparent between the veins in the magnesium-deficient treatments, while it was more uniformly distributed over the entire leaf in the potassium-deficient series. While the plants in the calcium-deficient series produced but little growth, they were not chlorotic. Treatments 67, 75, and 83, which contained little potassium or calcium and relatively large amounts of magnesium (fig. 1), produced little growth and were somewhat chlorotic. Significant differences between

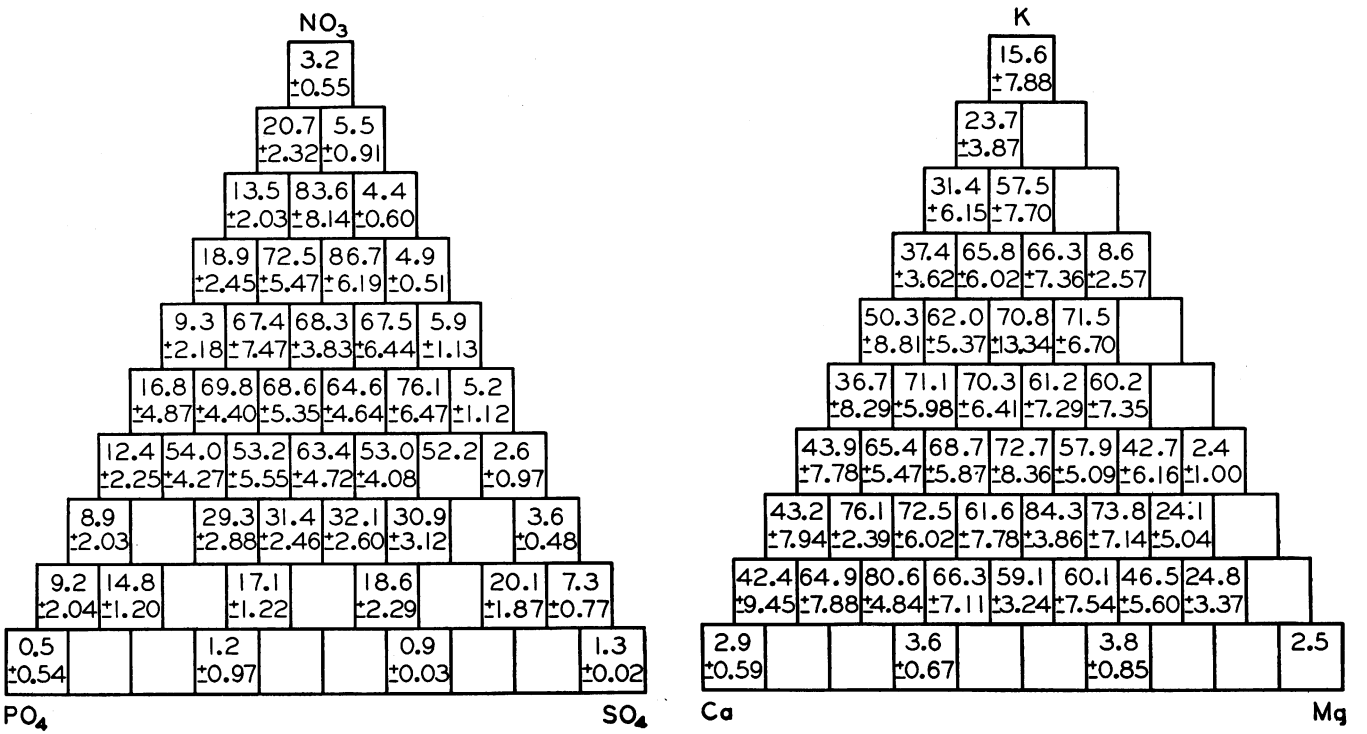

FIG. 2. Average fresh weight of turnip tops in sand cultures of first experiment giving treatment means (gm. per plant) together with the standard error. Each mean represents the average of eight plants.

Anion triangle (left). The "F" values from the variance analysis are: treatments 55.14† (43 degrees freedom); within blocks 2.20 (1 d.f.); and between blocks 3.69* (3 d.f.). No first order interactions were significant.

Cation triangle (right). The "F" values from the variance analysis are: treatments 10.58† (42 degrees of freedom); within blocks 1.56 (1 d.f.); and between blocks 5.76† (3 d.f.). No first order interactions were significant.

* Significant at the 5% level.

† Significant at the 1% level.

TABLE VIII

FRESH WEIGHTS, CAROTENE CONTENT, AND ASCORBIC ACID CONTENT OF TURNIPS GROWN IN SOIL OF EXPERIMENT I

TREATMENT	FRESH WT. OF TOPS (GM./PLANT) POT-CULTURES DUNKIRK	FRESH WT. OF TOPS (GM./PLANT) POT-CULTURES MARDIN	FRESH WT. OF TOPS (KG./9′ ROW) FIELD PLOTS DUNKIRK	FRESH WT. OF TOPS (KG./9′ ROW) FIELD PLOTS MARDIN	CAROTENE (μ/100 GM. FRESH WT. OF LEAVES) POT-CULTURES[1] DUNKIRK	CAROTENE (μ/100 GM. FRESH WT. OF LEAVES) POT-CULTURES[1] MARDIN	CAROTENE (μ/100 GM. FRESH WT. OF LEAVES) FIELD PLOTS[2] DUNKIRK	CAROTENE (μ/100 GM. FRESH WT. OF LEAVES) FIELD PLOTS[2] MARDIN	ASCORBIC ACID (MG./100 GM. FRESH WT. OF LEAVES) POT-CULTURES[3] DUNKIRK	ASCORBIC ACID (MG./100 GM. FRESH WT. OF LEAVES) POT-CULTURES[3] MARDIN	ASCORBIC ACID (MG./100 GM. FRESH WT. OF LEAVES) FIELD PLOTS[4] DUNKIRK	ASCORBIC ACID (MG./100 GM. FRESH WT. OF LEAVES) FIELD PLOTS[4] MARDIN
	gm.	*gm.*	*kg.*	*kg.*	μ	μ	μ	μ	*mg.*	*mg.*	*mg.*	*mg.*
1	19.9	6.1	.....	.....	60.5 ± 2.05	56.2 ± 9.10	....	....	288 ± 12.0	281 ± 13.1	....	....
2	23.8	18.1	0.57	0.47	58.8 ± 2.61	60.4 ± 5.63	64.7 ± 3.15	75.9 ± 4.18	273 ± 10.8	264 ± 7.7	195 ± 8.4	187 ± 17.1
3	19.6	.....	.....	.....	57.2 ± 3.36	....	....	....	292 ± 24.5	....	....	....
4	34.2	22.1	0.50	0.73	62.6 ± 8.59	51.8 ± 4.95	68.8 ± 3.08	75.9 ± 2.51	252 ± 9.0	259 ± 7.8	183 ± 4.9	182 ± 11.8
5	.....	.....	0.39	0.71	....	....	66.6 ± 2.49	72.8 ± 4.18	....	....	194 ± 14.2	170 ± 6.6
6	.....	.....	0.71	0.73	....	....	67.6 ± 3.29	74.9 ± 4.23	....	....	171 ± 4.5	173 ± 6.2
7	25.0	24.3	0.65	0.80	53.1 ± 5.69	69.8 ± 3.10	72.5 ± 1.93	74.5 ± 2.85	263 ± 22.7	287 ± 16.1	186 ± 7.4	171 ± 6.9
8	25.9	17.3	0.53	0.71	62.0 ± 5.04	58.8 ± 2.90	76.2 ± 2.00	76.3 ± 2.96	275 ± 19.2	260 ± 7.9	187 ± 7.3	186 ± 13.8
9	29.0	21.4	0.63	0.63	54.7 ± 4.17	58.0 ± 4.08	74.9 ± 3.84	82.3 ± 3.88	266 ± 13.0	296 ± 15.0	182 ± 9.9	192 ± 7.3
10	29.1	19.9	2.38	1.01	61.3 ± 5.02	63.8 ± 1.70	64.7 ± 4.27	77.1 ± 4.52	303 ± 19.6	270 ± 12.5	179 ± 7.6	197 ± 4.9
11	35.1	21.3	3.05	1.08	63.3 ± 3.42	46.5 ± 4.25	72.0 ± 3.65	73.9 ± 4.30	283 ± 13.2	265 ± 6.8	171 ± 12.6	170 ± 21.6
12	36.1	23.5	2.49	1.52	64.0 ± 2.63	64.6 ± 2.51	72.5 ± 2.26	78.3 ± 1.85	262 ± 12.2	267 ± 13.8	173 ± 9.6	191 ± 10.0
13	30.4	21.9	1.42	1.03	65.7 ± 2.76	66.9 ± 3.35	77.1 ± 6.65	82.0 ± 3.66	276 ± 20.1	306 ± 4.8	198 ± 10.5	185 ± 15.3
14	24.9	22.3	1.21	0.92	59.7 ± 12.08	68.3 ± 4.15	73.9 ± 2.55	74.7 ± 2.85	270 ± 6.9	265 ± 19.4	167 ± 10.6	179 ± 10.3
15	29.9	21.1	1.70	0.81	51.9 ± 6.15	61.1 ± 1.71	75.5 ± 4.32	77.5 ± 3.22	274 ± 17.7	269 ± 11.4	193 ± 11.6	172 ± 8.1
16	31.7	23.2	2.25	1.62	62.7 ± 5.79	52.7 ± 8.46	79.3 ± 2.68	75.6 ± 2.08	275 ± 14.9	282 ± 12.8	185 ± 19.0	187 ± 7.6
17	33.7	24.1	2.02	1.65	59.7 ± 3.94	53.8 ± 3.35	66.4 ± 1.10	81.1 ± 5.55	313 ± 9.4	277 ± 8.2	188 ± 11.4	191 ± 8.7
18	29.8	24.5	2.60	1.58	61.6 ± 5.19	63.6 ± 2.22	72.2 ± 3.78	82.2 ± 2.57	299 ± 15.1	276 ± 12.5	194 ± 7.8	190 ± 8.2
19	23.2	11.3	.....	.....	65.0 ± 4.58	62.8 ± 2.92	....	....	267 ± 7.9	267 ± 18.6	....	....
20	32.2	20.9	2.19	1.49	58.2 ± 11.88	60.5 ± 4.15	74.3 ± 1.58	76.6 ± 2.51	303 ± 3.5	271 ± 10.1	174 ± 21.8	189 ± 13.6
21	33.6	22.2	1.45	1.06	69.6 ± 1.42	56.2 ± 4.75	71.6 ± 1.10	74.3 ± 4.19	281 ± 6.2	269 ± 13.3	183 ± 4.3	190 ± 8.9
22	33.4	31.6	2.45	1.53	64.2 ± 2.22	53.3 ± 7.64	76.2 ± 2.28	82.9 ± 1.68	280 ± 12.5	247 ± 26.8	191 ± 7.2	192 ± 12.3
23	.....	.....	1.56	1.51	....	....	70.4 ± 2.67	79.1 ± 1.90	....	....	186 ± 10.3	179 ± 5.7
24	30.0	17.8	2.28	1.39	67.7 ± 3.95	67.3 ± 4.34	72.1 ± 3.84	72.4 ± 1.58	270 ± 13.5	277 ± 15.7	163 ± 14.3	185 ± 17.1
25	34.6	24.3	.....	.....	64.7 ± 2.26	51.2 ± 2.35	....	....	293 ± 7.6	246 ± 16.6	....	....
26	34.9	29.3	.....	.....	63.8 ± 4.19	51.6 ± 4.11	....	....	265 ± 12.3	288 ± 12.2	....	....
27	40.9	21.4	2.91	1.39	57.8 ± 5.27	51.9 ± 3.12	67.4 ± 4.18	70.5 ± 4.15	273 ± 12.5	259 ± 4.6	169 ± 16.6	162 ± 10.8
28	33.8	29.9	3.25	1.67	60.3 ± 3.69	54.0 ± 3.61	70.0 ± 2.97	71.0 ± 3.88	258 ± 10.1	240 ± 9.5	163 ± 10.4	172 ± 6.9
	Average vitamin values				61.2	58.5	71.6	76.6	278.2	270.3	181.5	182.2
20	Dunkirk soil in pots at fields				....	....	82.7 ± 0.92	86.7 ± 4.86	.....	.....	193 ± 6.8	215 ± 8.5
20	Mardin soil in pots at fields				....	....	70.2 ± 2.26	79.0 ± 2.71	.....	.....	220 ± 4.1	250 ± 10.2

"F" values for variance analyses are: (see above superscripts 1 to 4 incl.)

1. Treatments = 1.29, between blocks = 14.28,† soils = 3.93,* treatment × soils = 1.86.* No other first order interactions were significant.
2. Treatments = 2.22,† between blocks = 0.25, field = 30.47,† field × blocks = 8.70.† No other first order interactions were significant.
3. Treatments = 1.33, between blocks 11.22† soils = 3.05. No first order interactions were significant.
4. Treatments = 1.39, between blocks = 0.09, field = 1.26. No first order interactions were significant.

* Significant at the 5% level. † Significant at the 1% level.

blocks in both triangles are indicated since the "F" value in one case exceeds requirements for odds of 99:1 and in the other case satisfies the requirements for odds of 19:1. This indicates that variations in environmental conditions within the small experimental field produced significant effects upon growth. The field where the pots were located was adjacent to two buildings but was not shaded; however, there may have been some light reflection from the sides of the buildings to the field, and wind movements may have been modified.

Relatively great differences in growth were obtained as a result of soil treatment (table VIII). Differences in color and development were not as striking as between the sand culture treatments, although those treatments containing no phosphorus or excess lime produced plants, both in pots and in the field, which were a light green color, and many exhibited a mottled appearance. The more nearly optimum soil treatment produced plants of a deeper green color than those of the sand cultures. In general, those soil treatments involving nitrogen, phosphorus, or manure produced the most growth.

The average ascorbic acid values for the sand cultures each represent a total of eight analyses (fig. 3). Four of these analyses were made on June 30, and each of the four represented a composite sample taken from leaves of two plants in a particular block. The other four analyses were made from the same plants a few days later. The analysis of variance for the anion triangle shows that significant differences in ascorbic acid (at the 1 per cent. level) could be attributed to treatments, replications, and to replication-treatment and replication-date-of-harvest interactions. In the cation triangle, significant differences in ascorbic acid could be attributed to treatment and to replication and to a replication-date-of-harvest interaction. This would indicate that the treatments in both triangles produced significant effects upon ascorbic acid content as did also environmental variables associated with differences in location in the small experimental field. Differences with respect to date of harvest were associated with replication since a significant interaction was noted in both triangles. In the anion triangle, the replication-treatment interaction indicates an effect of treatment which is greater at certain locations than at others.

Those treatments which were deficient in sulfate, nitrate, and potassium produced plants which were significantly lower in ascorbic acid than the others, and there were increasing amounts of ascorbic acid with increasing nitrate supplies in the anion triangle in those treatments receiving less than 8.5 milliequivalents of nitrates per liter. One of the treatments deficient in calcium only produced plants of low ascorbic acid values while the other did not. Deficiencies of phosphorus and magnesium had little effect upon ascorbic acid content.

The differences in ascorbic acid content between the different replications noted here are in agreement with previous results obtained with tomatoes in which differences in ascorbic acid content could be associated with differ-

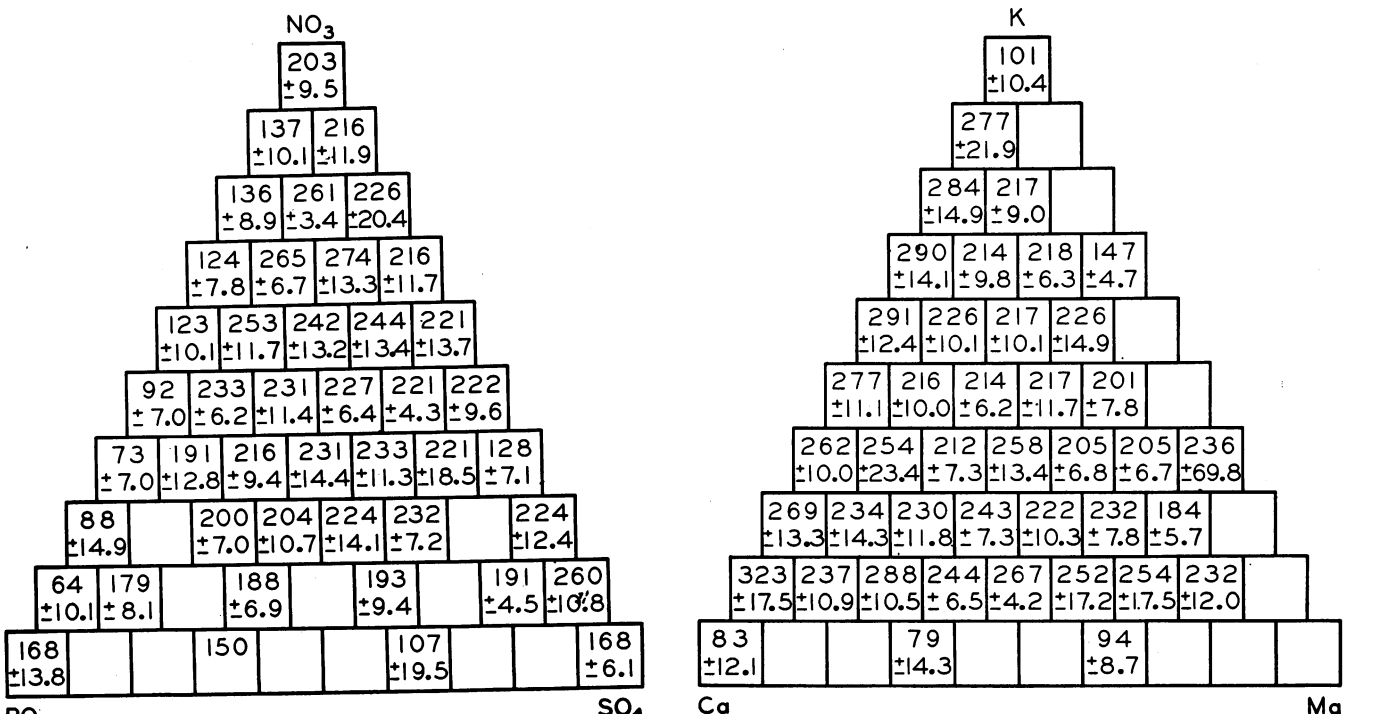

FIG. 3. Ascorbic acid values (mg. per 100 gm. fresh weight) of turnip greens of sand culture in first experiment giving treatment means with their standard errors.

Anion triangle (left). The "F" values from the variance analysis are: treatments 33.44* (43 degrees of freedom); between blocks 7.23* (1 d.f.); date of harvest 1.79 (1 d.f.); treatments × blocks 1.62* (129 d.f.); blocks × dates was not significant.

Cation triangle (right). The "F" values from the variance analysis are: treatments 19.93* (41 degrees of freedom); between blocks 9.98* (3 d.f.); date of harvest 1.17 (1 d.f.); blocks × date 4.85* (3 d.f.). No other first order interactions were significant.

* Significant at the 1% level.

ences in position in small fields. Significant differences in ascorbic acid content could be associated with replication interacting with the date of harvest indicating that significant variations in ascorbic acid content occurred within a short period of time in certain blocks.

From an analysis of variance of the data on ascorbic acid content of the plants in the pots containing soil, significant differences could be associated only between replications. There were no significant differences as a result of treatment, nor were there differences associated with the two types of soils used, nor were any interactions evidenced. In the field plots no significant differences were evident, indicating no effect of treatment or position in the field, nor were there differences between the two fields.

In general, the average carotene values (fig. 4, table VIII) given represent four analyses for any given treatment. Each analysis was obtained from a combined sample of two plants in any given block. The analysis of variance demonstrated significant differences in both the anion and cation triangles of the sand cultures associated with treatment, and no significant differences between replications were noted. The treatments deficient in sulfate, magnesium, potassium, and calcium were significantly lower in carotene than the other treatments, and increasing amounts of carotene were obtained with increasing supplies of nitrate in those treatments containing less than 11.3 milliequivalents of nitrate per liter. The treatments deficient in phosphate contained as much carotene as any of the other treatments of the anion triangle. Low values for carotene were obtained in treatments 75 and 83 (fig. 1) which also produced poor growth. All plants which were significantly lower in carotene were also chlorotic. While those plants growing on the phosphorus-deficient treatments produced relatively little growth, the leaves were of a very deep green color and rich in carotene. This would indicate that the carotene content is not necessarily directly correlated with the amount of growth produced since these phosphorus-deficient plants produced less than one-tenth the amount of growth produced in some of the more optimum treatments of the same carotene content, and less than one-third the growth of some of the magnesium-deficient series which had much less carotene.

In the soil-pot cultures, significant differences in carotene could be attributed to differences between the replications, but not to treatment. Differences which could be correlated with the soils used were significant at the 5 per cent. level, and the treatment-soil interaction was also significant at the 5 per cent. level indicating that possible effects of treatment were dependent upon the soil used. In the field plots significant differences in carotene could be attributed to treatment, and significant differences were also apparent between the two fields. A field-replication interaction is also highly significant, indicating that the effects due to replication are greater in one field than in the other. The plants at the Dunkirk field were lower in carotene than at the Mardin field. The values for carotene at the fields range from 64.7 to 79.3 and from 70.5 to 82.9 gamma per 100 grams, respec-

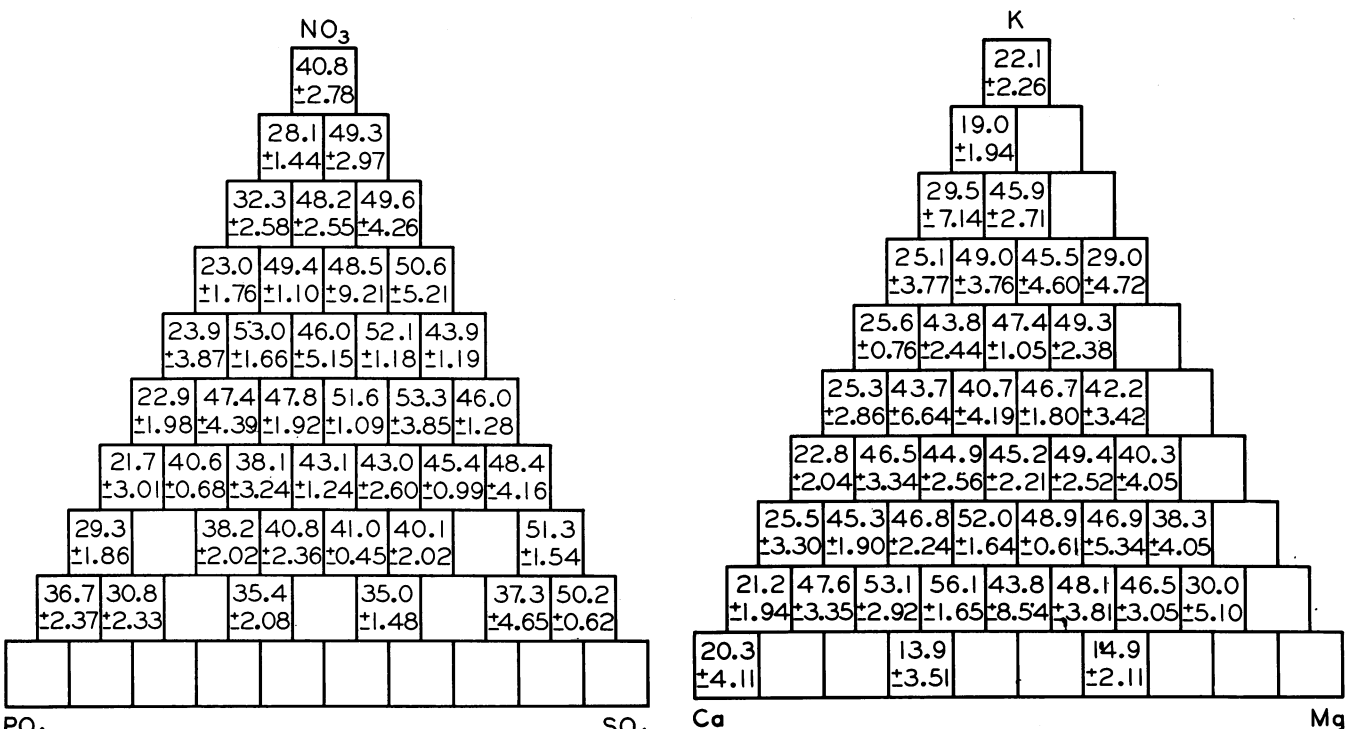

FIG. 4. Carotene values (mg./100 gm. fresh wt.) of turnip greens of sand cultures in first experiment giving treatment means with their standard error.

Anion triangle (left). The "F" values from the variance analysis are: treatments 8.82* (39 degrees of freedom); between blocks 0.87 (3 d.f.).

Cation triangle (right). The "F" values from the variance analysis are: treatments 10.98* (40 degrees of freedom); between blocks 2.48 (3 d.f.).

* Significant at the 1% level.

tively. While these differences which may be attributed to treatments and to fields are significant statistically, it seems that they have little practical importance. The differences between the plants at the laboratory and those at the two field locations are much greater than the differences between the two fields or between treatments in either field.

At each of the field locations, ten pot-cultures of each soil were established corresponding to treatment 20, table VII. These cultures were placed alongside of each field and served as a check on the effects of location. The results obtained with these cultures are given at the bottom of table VIII. The ascorbic acid values of the plants in these cultures are slightly higher than the values obtained in the field plants, but all are considerably lower than the values obtained in the pot-cultures at the laboratory. The differences between these pot-cultures and those at the laboratory indicate an appreciable effect of location or time of harvest on ascorbic acid values.

The carotene values of the plants in the pot-cultures at each field closely approximated the average values obtained in the field-grown plants. As mentioned, the differences in the carotene values between the two fields were statistically significant, and both fields gave values considerably higher than those obtained in the pot-cultures and sand cultures at the laboratory. As was the case with the ascorbic acid values, it seems apparent that variations in carotene may be correlated with time of harvest or location. In fact, all these results on carotene and ascorbic acid demonstrate that factors which were not under particular control in this experiment produced much greater variations in the vitamin content than was caused by the various treatments employed. This is particularly apparent in a comparison of the carotene and ascorbic acid values for the sand and the soil-pot-cultures, which were grown in alternating blocks at the laboratory, but analyzed on different days. As will be discussed in greater detail later, these variations in the average values obtained in the various sets of plants were apparently associated with certain differences in climate correlated with location or date of harvest.

Experiment II

Since Experiment I was carried out at a time equivalent to the spring crops, a second experiment was conducted to correspond to the fall crop, in order to amplify the results of the first. Many of the plants in the field of the first experiment sent up flower stalks, but in the second experiment they did not. The sand culture treatments were modified to include some in which the plants received nutrient solutions containing all the macro-elements, but in a greater degree of unbalance than those used previously. The plants were harvested at an earlier stage of growth than that at which they were harvested in the first.

Procedure

Seeds of the same variety were planted on August 7. The same procedures of handling the seedlings were followed as in the first experiment. The seedlings for the sand cultures were transplanted on August 26, and the

seedlings in the soil were thinned on September 4. The plants in sand cultures were started upon their respective nutrient treatments immediately after transplanting. The soil treatments were essentially the same as those of Experiment I, except that a few treatments were added to the pot-cultures and two treatments were eliminated. In most cases no more fertilizers were added to the soil. Only the nitrogen fertilizer was reapplied in original amounts. In the field the plots were cultivated lightly and the seeds planted without further treatment with the exception that a reapplication of manure was made on two of the four manured plots. In the pots containing soil additional treatments consisted of applications of various organic materials. The soil treatments are listed in table IX and the nutrient composition of the sand cultures shown in tables X and XI. The samples were collected and analyses made in a manner similar to that of Experiment I. Standard errors were computed for the data of each treatment where possible, but the analysis of variance was not made.

Results

The plants grew rapidly, especially after the transplanting and thinning dates. In general, the plants in the soil were of a greener color than were the plants in the sand cultures, although all the plants except those on deficiency treatments were of an even greenness. On September 24 (one day before samples were taken for the first vitamin analysis) careful observations were made on all of the plants. At the time of harvest, data similar to that of the first experiment were collected. Only the data of the fresh weights of the tops are included here. As in Experiment I, all observations served to demonstrate a great effect of the treatments on the growth and development of the plants, and differences in appearance associated with mineral deficiencies were similar in both experiments.

The ascorbic acid content of the plants, tables X and XI, in the sand cultures of this experiment were relatively much lower than were those of the first experiment, and the variations which might be correlated with treatment were relatively slight. Low ascorbic acid values were obtained in treatments deficient in sulfate and potassium, and high ascorbic acid values could be correlated with treatments relatively high in magnesium; i.e., 85, 86, and 87. Treatments 70, 71, and 72, which were relatively low in calcium and relatively high in magnesium, also seemed to produce high ascorbic acid values. These latter six treatments are not comparable to any of the treatments of Experiment I. In contrast to Experiment I, treatment 50, which was deficient in calcium, resulted in high ascorbic acid values. Also in contrast to Experiment I, there was no correlation between nitrogen supply and ascorbic acid content with the single exception that treatment 13, which was deficient in nitrates, produced plants relatively high in ascorbic acid. Treatments 42, 43, and 44, which received additional supplies of nitrogen in the form of ammonium nitrate, had ascorbic acid values comparable to the average values obtained in both triangles.

TABLE IX

FERTILIZER TREATMENTS APPLIED TO SOIL-POTS AND FIELD PLOTS OF EXPERIMENT II

TREATMENT	LIME PER ACRE	INORGANIC FERTILIZER PER ACRE						APPLICATION TO†				REMARKS
								POT-CULTURES		FIELD PLOTS		
		Ca	K	Mg	N	P_2O_5	S	DUNKIRK	MARDIN	DUNKIRK	MARDIN	
	tons	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*					
1*	.....	.....	.....	.....	.....	.....	.....	+	+	−	−	
2	2	.....	.....	.....	.....	.....	.....	+	+	−	−	Ash of 10 tons of alfalfa per acre applied
3	2	.....	.....	.....	.....	.....	.....	+	+	−	−	Ash of 10 tons of alfalfa per acre applied
4*	10	.....	.....	.....	.....	.....	.....	+	+	+	+	
5*	10	.....	.....	.....	.....	.....	.....	−	−	+	+	Added microelements, residual
6*	2	.....	.....	.....	.....	.....	.....	−	−	+	+	Added microelements, residual
7*	2	29.6	43.6	13.5	20.5	.....	29.7	+	+	+	+	
8*	2	29.6	43.6	13.5	.....	.....	59.3	+	+	+	+	
9*	2	29.6	43.6	13.5	41.0	.....	.....	+	+	+	+	
10*	2	29.6	43.6	13.5	.....	105.0	29.7	+	+	+	+	
11*	2	29.6	43.6	13.5	.....	210.0	.....	+	+	+	+	
12*	2	29.6	43.6	13.5	20.5	105.0	.....	+	+	+	+	
13*	2	37.0	.....	22.5	16.4	63.6	17.8	+	+	+	+	
14*	2	.....	.....	45.0	16.4	63.6	17.8	+	+	+	+	
15*	2	74.0	.....	.....	16.4	63.6	17.8	+	+	+	+	
16*	2	37.0	72.0	.....	16.4	63.6	17.8	+	+	+	+	
17*	2	.....	144.0	.....	16.4	63.6	17.8	+	+	+	+	
18*	2	.....	72.0	22.5	16.4	63.6	17.8	+	+	+	+	

TABLE IX—*Continued*

Treatment	Lime per acre	Inorganic fertilizer per acre						Application to†				Remarks
								Pot-cultures		Field plots		
		Ca	K	Mg	N	P_2O_5	S	Dunkirk	Mardin	Dunkirk	Mardin	
	tons	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*					
19*	.....	24.7	48.0	15.0	16.4	63.6	17.8	+	+	−	−	
20*	2	24.7	48.0	15.0	16.4	63.6	17.8	+	+	+	+	
21*	2	24.7	48.0	15.0	16.4	63.6	17.8	+	+	+	+	Added microelements, residual
22*	10	24.7	48.0	15.0	16.4	63.6	17.8	+	+	+	+	
23*	10	24.7	48.0	15.0	16.4	63.6	17.8	−	−	+	+	Added microelements, residual
24*	2	29.6	43.6	13.5	13.6	70.0	19.8	+	+	+	+	
25	2	.....	.....	.....	.....	.....	.....	+	+	−	−	10 tons alfalfa per acre applied
26	2	.....	.....	.....	.....	.....	.....	+	+	−	−	10 tons straw per acre applied
27*	2	.....	.....	.....	.....	.....	.....	−	−	+	+	10 tons manure per acre, residual
28*	2	24.7	48.0	15.0	16.4	63.6	17.8	−	−	+	+	10 tons manure per acre, residual
29	2	.....	.....	.....	.....	.....	.....	−	−	+	+	10 tons manure per acre, residual and applied
30	2	24.7	48.0	15.0	16.4	63.6	17.8	−	−	+	+	10 tons manure per acre, residual and applied
31	2	.....	.....	.....	.....	.....	.....	+	+	−	−	Soil puddled
32	2	.....	.....	.....	.....	.....	.....	+	+	−	−	10 tons straw per acre + N to equal C: N of 14: 1
33	2	.....	.....	.....	.....	.....	.....	+	−	−	−	Turnips transplanted to pots 3 weeks after germination.

* Treatments same as in Expt. I. Fertilizer treatments residual, with the exception of nitrogen, which was supplied in the same amounts as at the original application.

† The symbol + indicates that the fertilizers to the left have been added.

The carotene content of the leaves was low in treatments deficient in sulfate (treatment 3), magnesium (treatment 47), and potassium (treatment 57), which corresponds closely to the results of the first experiment. There are indications in the first experiment that treatments deficient in calcium produced plants low in carotene, but such was not the case in the second

TABLE X

COMPOSITION OF NUTRIENT SOLUTIONS, FRESH WEIGHTS, ASCORBIC ACID, AND CAROTENE CONTENTS OF PLANTS OF THE ANION TRIANGLE OF EXPERIMENT II

TREATMENT	COMPOSITION OF NUTRIENTS IN M. EQ./LITER*				FRESH WT. OF TOPS	ASCORBIC ACID (MG./100 GM.)	CAROTENE (μ/GM.)
	NO_3	H_2PO_4	SO_4	NH_4			
	m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*gm.*	*mg.*	μ
1	19.83	2.83	2.83	.....	52 ± 3.3	153 ± 1.9	43.7 ± 3.12
2	14.17	5.67	5.67	.....	37 ± 4.9	145 ± 3.3	37.2 ± 3.99
3	12.75	12.75	0.00	.....	12 ± 2.1	111 ± 6.6	19.7 ± 5.84
4	11.33	11.33	2.83	.....	32 ± 2.3	159 ± 2.2	38.9 ± 1.71
5	11.33	2.83	11.33	.....	32 ± 4.3	161 ± 3.4	32.5 ± 8.37
6	12.75	0.00	12.75	.....	8 ± 1.6	194 ± 4.7	56.1 ± 11.83
7	8.50	8.50	8.50	.....	21 ± 3.8	131 ± 2.0	37.2 ± 3.56
8	5.67	14.17	5.67	.....	18 ± 2.2	162 ± 3.0	42.7 ± 9.14
9	5.67	5.67	14.17	.....	18 ± 2.0	166 ± 5.1	37.0 ± 7.27
10	2.83	19.83	2.83	.....	10 ± 1.6	163 ± 2.1	21.9 ± 1.48
11	2.83	11.33	11.33	.....	9 ± 1.6	152 ± 2.1	34.2 ± 5.70
12	2.83	2.83	19.83	.....	9 ± 1.4	162 ± 1.2	.....
13	0.00	12.75	12.75	.....	1 ± 0.3	195 ± 4.3	34.7 ± 7.56
14	16.04	8.02	1.42	.....	36 ± 3.2	162 ± 3.6	21.4 ± 4.89
15	16.52	8.26	0.71	.....	38 ± 3.8	148 ± 2.3	44.2 ± 8.98
16	16.76	8.38	0.35	.....	31 ± 3.1	135 ± 1.4	40.5 ± 3.52
17	8.02	16.04	1.42	.....	18 ± 9.6	155 ± 4.0	24.8 ± 8.29
18	8.26	16.52	0.71	.....	26 ± 2.3	153 ± 2.4	16.6 ± 6.76
19	8.38	16.76	0.35	.....	22 ± 3.0	140 ± 2.8	31.8 ± 3.94
20	8.02	1.42	16.04	.....	25 ± 9.8	157 ± 4.1	30.2 ± 0.00
21	8.26	0.71	16.52	.....	23 ± 2.8	160 ± 4.4	40.5 ± 7.69
22	8.38	0.35	16.76	.....	21 ± 2.7	150 ± 8.3	40.0 ± 3.56
23	16.04	1.42	8.02	.....	36 ± 2.2	157 ± 1.6	42.3 ± 0.79
24	16.52	0.71	8.26	.....	42 ± 3.4	165 ± 2.7	42.7 ± 7.72
25	16.76	0.35	8.36	.....	28 ± 2.3	174 ± 3.6	42.5 ± 5.78
26	24.08	0.71	0.71	.....	39 ± 5.9	151 ± 2.1	40.6 ± 5.34
27	21.96	2.83	0.71	.....	49 ± 2.4	158 ± 2.3	36.9 ± 4.70
28	21.96	1.77	1.77	.....	48 ± 10.1	145 ± 2.6	41.3 ± 4.45
29	21.96	0.71	2.83	.....	40 ± 3.2	165 ± 2.6	44.9 ± 3.95
30	0.71	24.08	0.71	.....	3 ± 0.7	150 ± 3.4	28.6 ± 5.34
31	2.83	21.96	0.71	.....	8 ± 0.7	154 ± 3.1	32.6 ± 4.14
32	1.77	21.96	1.77	.....	3 ± 1.0	157 ± 1.8	39.9 ± 7.86
33	0.71	21.96	2.83	.....	3 ± 0.3	117 ± 0.5	42.4 ± 9.25
34	0.71	0.71	24.08	.....	3 ± 0.3	165 ± 2.0	19.9 ± 4.47
35	2.83	0.71	21.96	.....	12 ± 1.5	165 ± 2.3	27.2 ± 2.13
36	1.77	1.77	21.96	.....	6 ± 0.7	175 ± 1.3	33.4 ± 5.32
37	0.71	2.83	21.96	.....	4 ± 0.5	161 ± 9.5	18.9 ± 0.87
38	8.50	8.50	8.50	.....	29 ± 3.0	159 ± 3.3	25.9 ± 5.10
39	1.42	12.04	12.04	.....	6 ± 1.2	166 ± 6.3	37.4 ± 3.90
40	0.71	12.39	12.39	.....	3 ± 0.5	164 ± 5.7	19.1 ± 2.72
41	0.35	12.57	12.57	.....	2 ± 0.3	150 ± 3.7	31.5 ± 9.71
42	12.75	8.50	8.50	4.25	59 ± 4.6	152 ± 1.9	31.8 ± 3.85
43	17.00	8.50	8.50	8.50	77 ± 4.8	169 ± 2.5	37.9 ± 5.76
44	21.25	8.50	8.50	12.75	69 ± 15.8	151 ± 2.2	48.6 ± 13.78

* Each of these nutrient solutions contained the following milliequivalents per liter of cations: Ca = 12.0, K = 4.5, and Mg = 9.0.

experiment. Treatments 60 and 63 in which only 0.35 milliequivalents of magnesium were used also produced plants low in carotene. It may be noted that the average carotene values were very near the same in both experiments.

TABLE XI

COMPOSITION OF NUTRIENT SOLUTIONS, FRESH WEIGHTS, ASCORBIC ACID, AND CAROTENE CONTENTS OF PLANTS OF THE CATION TRIANGLE OF EXPERIMENT II

TREATMENT	COMPOSITION OF NUTRIENTS IN M. EQ./LITER*			FRESH WT. OF TOPS	ASCORBIC ACID (MG./100 GM.)	CAROTENE (μ/GM.)
	K	Ca	Mg			
	m. eq.	*m. eq.*	*m. eq.*	*gm.*	*mg.*	μ
45	19.83	2.83	2.83	28 ± 2.1	134 ± 2.1	32.5 ± 2.2
46	14.17	5.67	5.67	26 ± 5.5	137 ± 2.7	33.6 ± 6.7
47	12.75	12.75	0.00	17 ± 1.9	174 ± 3.8	23.5 ± 2.2
48	11.33	11.33	2.83	34 ± 3.6	163 ± 3.3	35.3 ± 2.6
49	11.33	2.83	11.33	34 ± 2.6	152 ± 4.3	35.5 ± 5.0
50	12.75	0.00	12.75	0.3 ± 0.27	212 ± 8.3	32.3 ± 14.6
51	8.50	8.50	8.50	37 ± 2.4	145 ± 3.1	37.4 ± 0.0
52	5.67	14.17	5.67	39 ± 4.3	150 ± 2.3	27.2 ± 4.8
53	5.67	5.67	14.17	30 ± 3.6	144 ± 3.7	40.7 ± 4.3
54	2.83	19.83	2.83	32 ± 3.4	158 ± 2.5	43.9 ± 2.2
55	2.83	11.33	11.33	33 ± 3.4	162 ± 3.5	42.9 ± 5.2
56	2.83	2.83	19.83	14 ± 3.9	156 ± 4.4	32.4 ± 6.0
57	0.00	12.75	12.75	6 ± 1.3	87 ± 3.2	23.5 ± 3.4
58	16.04	8.02	1.42	31 ± 4.0	146 ± 2.6	39.4 ± 5.0
59	16.52	8.26	0.71	33 ± 2.9	144 ± 2.8	31.5 ± 0.7
60	16.76	8.38	0.35	30 ± 0.9	140 ± 2.6	25.4 ± 6.5
61	8.02	16.04	1.42	37 ± 2.8	158 ± 2.8	40.9 ± 4.3
62	8.26	16.52	0.71	39 ± 5.7	161 ± 3.6	33.5 ± 3.3
63	8.38	16.76	0.35	35 ± 4.4	161 ± 5.9	25.7 ± 5.0
64	1.42	8.02	16.04	18 ± 2.4	174 ± 3.9	45.3 ± 6.0
65	0.71	8.26	16.52	19 ± 1.8	162 ± 1.3	45.0 ± 3.3
66	0.35	8.38	16.76	14 ± 2.4	125 ± 2.2	33.6 ± 8.1
67	1.42	16.04	8.02	30 ± 3.6	173 ± 3.3	25.1 ± 8.9
68	0.71	16.52	8.26	24 ± 2.5	136 ± 2.7	45.0 ± 3.4
69	0.35	16.76	8.38	18 ± 1.8	138 ± 3.5	36.9 ± 6.1
70	8.02	1.42	16.04	3 ± 0.8	166 ± 8.7	21.1 ± 1.0
71	8.26	0.71	16.52	4 ± 2.3	194 ± 7.9	33.0 ± 0.0
72	8.38	0.35	16.76	2 ± 1.2	188 ± 2.4	14.2 ± 3.0
73	16.04	1.42	8.02	19 ± 2.3	139 ± 2.2	33.1 ± 6.5
74	16.52	0.71	8.26	12 ± 3.3	145 ± 3.6	39.5 ± 7.4
75	16.76	0.35	8.36	3 ± 0.4	157 ± 3.6	27.7 ± 7.3
76	24.08	0.71	0.71	19 ± 2.5	123 ± 3.5	27.7 ± 4.0
77	21.96	2.83	0.71	37 ± 1.2	126 ± 2.7	30.3 ± 1.0
78	21.96	1.77	1.77	35 ± 6.8	133 ± 3.5	21.3 ± 4.3
79	21.96	0.71	2.83	18 ± 3.1	125 ± 2.6	29.2 ± 5.9
80	0.71	24.08	0.71	19 ± 1.7	151 ± 3.1	36.8 ± 1.8
81	2.83	21.96	0.71	29 ± 5.5	182 ± 2.8	40.7 ± 2.8
82	1.77	21.96	1.77	26 ± 1.7	177 ± 2.9	42.4 ± 0.7
83	0.71	21.96	2.83	24 ± 3.3	160 ± 5.1	47.9 ± 5.5
84	0.71	0.71	24.08	0.5 ± 0.51	.....	.....
85	2.83	0.71	21.96	8 ± 6.5	214 ± 150.9	.....
86	1.77	1.77	21.96	1 ± 0.3	282 ± 4.1	55.2 ± 0.0
87	0.71	2.83	21.96	4 ± 1.4	243 ± 5.5	31.1 ± 6.8

* Each of these nutrient solutions contained the following milliequivalents per liter of anions: $NO_3 = 12.0$, $H_2PO_4 = 4.5$, and $SO_4 = 9.0$.

Treatments 1, 42, 43, and 44 in which the largest amounts of nitrogen were used were richer in carotene than average; it seems apparent that in both experiments carotene content could be directly correlated with nitrogen sup-

ply. It was also evident that treatments deficient in or relatively low in phosphates produced plants high in carotene. The chlorophyll values were somewhat correlated with the carotene values, although the results are rather variable and have not been included. The chlorophyll content seemed to be relatively high in plants given treatments deficient in phosphate or rich in nitrogen.

Comparisons may be made between the amount of growth of the tops as measured by fresh weight and the vitamin values. There was almost a direct correlation between the growth in the anion triangle and the amount of nitrogen supplied, although treatments 3 and 6, which were deficient in sulfate and phosphate, respectively, showed little growth. The amount of growth varied from 1 to 29 grams per plant. In the cation triangle, decreases in growth could be correlated with amounts of potassium of 0.71 milliequivalents or less and with amounts of calcium of 1.42 milliequivalents or less. Amounts of magnesium as low as 0.35 milliequivalents resulted in nearly the optimum amount of growth, although growth was decreased appreciably when magnesium was not present in the nutrient solution. Neither the percentage of ascorbic acid nor carotene showed any close correlation with the amount of growth produced. For example, plants from treatments deficient in phosphorus were as high or higher in both ascorbic acid and carotene than plants from most of the other treatments, although very little growth was obtained. While the nitrogen supply produced greater effects on growth than any other nutrient available, there was little correlation between nitrogen supply and ascorbic acid content, and the carotene content varied but slightly. Treatments 42, 43, and 44, which produced by far the greatest amount of top growth of any of the treatments, had average ascorbic acid values, and the carotene values were only slightly above average.

In the soil treatment the response to treatment was less marked in terms of growth (table XII) in this experiment than in the spring crop, probably because most of the fertilizer treatments were not repeated. The treatments where organic material or ash was applied, however, produced exceptionally good growth.

The only effect of treatment on vitamin content was in treatment 34 (alfalfa incorporated) where an increase in carotene[3] was accompanied by a decrease in ascorbic acid.[4] The data from the sand-culture experiments would not indicate that this effect was due to nitrogen supply.

The ascorbic acid values for the plants grown in soil-pot-cultures were lower than in the first experiment (cf. sand cultures) while the values for plants grown at the fields were higher than in Experiment I. In the second experiment, the ascorbic acid content of the field plants was higher than those of the soil-pot-cultures which were also higher than those for the sand cultures. The carotene values for the second experiment, however, are

[3] Significant at 5 per cent. level in a "T" test.

[4] Significant at 5 per cent. level in a "T" test.

TABLE XII

FRESH WEIGHT, CAROTENE CONTENT, AND ASCORBIC ACID CONTENT OF TURNIPS GROWN IN SOIL, EXPERIMENT II

TREATMENT	FRESH WEIGHT OF TOPS (GM./PLANT) POT-CULTURES DUNKIRK	FRESH WEIGHT OF TOPS (GM./PLANT) POT-CULTURES MARDIN	FRESH WEIGHT OF TOPS (KG./9′ ROW) FIELD PLOTS DUNKIRK	FRESH WEIGHT OF TOPS (KG./9′ ROW) FIELD PLOTS MARDIN	CAROTENE (μ/100 GM. FRESH WT. OF LEAVES) POT-CULTURES DUNKIRK	CAROTENE (μ/100 GM. FRESH WT. OF LEAVES) POT-CULTURES MARDIN	CAROTENE (μ/100 GM. FRESH WT. OF LEAVES) FIELD PLOTS DUNKIRK	CAROTENE (μ/100 GM. FRESH WT. OF LEAVES) FIELD PLOTS MARDIN	ASCORBIC ACID (MG./100 GM. FRESH WT. OF LEAVES) POT-CULTURES DUNKIRK	ASCORBIC ACID (MG./100 GM. FRESH WT. OF LEAVES) POT-CULTURES MARDIN	ASCORBIC ACID (MG./100 GM. FRESH WT. OF LEAVES) FIELD PLOTS DUNKIRK	ASCORBIC ACID (MG./100 GM. FRESH WT. OF LEAVES) FIELD PLOTS MARDIN
	gm.	*gm.*	*kg.*	*kg.*	μ	μ	μ	μ	*mg.*	*mg.*	*mg.*	*mg.*
1	6.3	1.0	.....	.....	.....	.....	.....	.....	185 ± 5.3	168 ± 14.7	.....	.....
2	9.6	5.2	0.46	0.12	45.1 ± 5.00	31.7 ± 5.90	37.8 ± 1.59	40.2 ± 5.38	177 ± 7.7	187 ± 8.6	202 ± 10.6	234 ± 9.5
3	5.7	7.3	.....	.....	.....	.....	.....	.....	170 ± 5.9	172 ± 10.4	.....	.....
4	10.0	10.3	0.28	0.16	55.0 ± 1.37	29.7 ± 7.85	.....	.....	178 ± 4.2	198 ± 27.9	222 ± 7.1	203 ± 5.4
5	.....	.....	0.56	0.24	.....	.....	.....	.....	.....	.....	192 ± 7.2	218 ± 22.3
6	.....	.....	0.42	0.21	.....	.....	.....	.....	.....	.....	226 ± 9.9	216 ± 10.9
7	7.4	7.5	0.52	0.24	.....	.....	.....	.....	180 ± 7.4	206 ± 5.3	218 ± 7.8	222 ± 18.5
8	6.7	5.5	0.34	0.20	.....	.....	.....	.....	178 ± 7.9	178 ± 10.1	207 ± 14.6	206 ± 13.8
9	11.0	10.4	0.41	0.27	.....	.....	.....	.....	178 ± 10.9	170 ± 8.9	190 ± 6.4	233 ± 8.9
10	8.9	6.3	0.50	0.28	.....	.....	.....	.....	186 ± 11.4	214 ± 11.7	200 ± 7.3	209 ± 6.7
11	10.4	5.5	0.88	0.18	.....	.....	.....	.....	185 ± 9.0	182 ± 4.2	191 ± 8.3	237 ± 17.8
12	10.6	10.3	0.70	0.37	.....	.....	.....	.....	191 ± 8.8	191 ± 4.0	216 ± 15.0	204 ± 10.4
13	8.9	6.8	0.57	0.31	.....	.....	.....	.....	162 ± 12.6	196 ± 10.8	196 ± 5.7	214 ± 14.6
14	9.4	7.9	0.85	0.21	.....	.....	.....	.....	185 ± 8.8	213 ± 10.3	181 ± 6.7	235 ± 13.5
15	10.1	6.1	0.54	0.25	.....	.....	49.2 ± 2.12	45.8 ± 2.63	179 ± 5.0	195 ± 10.2	200 ± 6.7	236 ± 8.1
16	7.2	6.7	0.42	0.32	.....	.....	.....	.....	170 ± 4.7	212 ± 8.8	203 ± 10.7	187 ± 7.2
17	10.8	6.5	0.64	0.45	.....	.....	.....	.....	181 ± 3.6	152 ± 18.0	199 ± 9.1	214 ± 11.9
18	10.4	9.1	0.56	0.33	.....	.....	.....	.....	175 ± 6.5	190 ± 9.4	203 ± 7.1	200 ± 8.5
19	6.5	2.6	.....	.....	.....	.....	.....	.....	180 ± 6.7	208 ± 24.6	.....	.....
20	10.8	8.2	0.47	0.26	37.2 ± 7.43	32.4 ± 6.11	39.1 ± 5.22	49.0 ± 3.80	179 ± 6.4	187 ± 7.7	206 ± 14.4	196 ± 11.1
21	11.6	8.3	0.54	0.28	29.0 ± 4.46	36.6 ± 3.05	.....	.....	190 ± 9.0	194 ± 9.2	213 ± 10.5	206 ± 9.6
22	10.0	9.5	0.66	0.33	36.0 ± 3.25	42.6 ± 5.58	.....	.....	188 ± 5.3	168 ± 6.0	215 ± 10.6	216 ± 14.0
23	.....	.....	0.70	0.33	.....	.....	36.6 ± 4.77	45.3 ± 3.03	.....	.....	202 ± 9.7	188 ± 5.4
24	8.2	6.5	0.40	0.28	41.2 ± 6.50	38.0 ± 8.66	38.6 ± 3.34	50.4 ± 0.56	164 ± 7.4	182 ± 7.7	206 ± 5.7	238 ± 7.9
25	12.7	22.8	.....	.....	.....	.....	.....	.....	169 ± 2.6	190 ± 7.4	.....	.....
26	16.3	28.9	.....	.....	33.3 ± 4.02	40.0 ± 3.37	.....	.....	182 ± 7.6	188 ± 11.9	.....	.....
27	.....	.....	0.75	0.32	.....	.....	.....	.....	.....	.....	200 ± 11.6	202 ± 11.3
28	.....	.....	0.82	0.51	.....	.....	.....	.....	.....	.....	203 ± 12.9	203 ± 3.9
29	.....	.....	1.57	0.73	.....	.....	42.1 ± 5.04	46.0 ± 2.66	.....	.....	160 ± 4.5	183 ± 9.3
30	.....	.....	1.80	0.74	.....	.....	.....	.....	.....	.....	155 ± 7.5	184 ± 12.4
31	13.2	16.1	.....	.....	33.2 ± 4.00	49.7 ± 5.39	.....	.....	182 ± 6.9	168 ± 8.0	.....	.....
32	26.1	23.6	.....	.....	.....	.....	.....	.....	170 ± 12.7	156 ± 8.4	.....	.....
33	20.1	.....	.....	.....	.....	.....	.....	.....	183 ± 11.0	.....	.....	.....
34	28.4	32.8	.....	.....	64.3 ± 4.10	64.0 ± 16.25	.....	.....	144 ± 2.5	142 ± 5.6	.....	.....
	Average vitamin values				41.6	40.5	40.6	46.1	177	185	200	211
20	Dunkirk soil in pots at fields				.....	.....	39.7 ± 3.60	51.5 ± 8.06	.....	.....	211 ± 6.3	227 ± 5.6
20	Mardin soil in pots at fields				.....	.....	19.9 ± 3.46	36.9 ± 5.30	.....	.....	221 ± 8.6	221 ± 4.5

essentially the same for plants grown in field plots, soil-pot-cultures, and sand cultures.

Discussion

With respect to the ascorbic acid content of turnip greens, both of these experiments indicate that the differences in environment of one location and another or from one date to another have a marked effect upon the ascorbic acid content. Just which factors were responsible for the variations which occurred between locations in these experiments is unknown. In the original organization of the work, emphasis was placed upon the expected variations in ascorbic acid between treatments, and it seemed logical to analyze all of the plants from one location on the same day, and therefore no separation of the effects of location and date of harvest is possible. In certain of the data, it is possible to determine which differences were associated with date of harvest, and while these are statistically significant, they are of relatively less magnitude than the differences between locations.

The plants grown in soil showed no indication that fertilizer treatment influenced ascorbic acid content to any great extent, in spite of the fact that the fertilizer treatments employed had a marked effect upon the growth of the plants. In the sand cultures, significant effects of treatments on vitamin content were produced in certain cases where the treatments were deficient in one or more nutrient elements, but in all these cases very little growth of the plants was obtained. Increasing nitrogen supplies seemed to result in increasing ascorbic acid values in the first experiment, while in the second no appreciable effect of nitrogen supply was noted. In the first experiment, conducted in the early summer, the average ascorbic acid values were much higher than in the second. In the preliminary experiments with plants produced in the greenhouse during the winter, the average ascorbic acid values were approximately half those obtained in the experiments conducted during the summer. The growth of the plants during the winter months was not as rapid as during the summer, but at the time the plants were harvested in the preliminary work they were of about the same general appearance as those harvested during the summer. Thus, the average ascorbic acid values obtained in experiments at various seasons differed greatly.

In a review of the literature, MAYNARD and BEESON (**10**) found considerable disagreement among the various investigators as to whether or not fertilizer treatments affected the ascorbic acid content of different varieties of plants. Many investigators claimed no effect of fertilizer treatment; others claimed that nitrogen fertilization increased ascorbic acid content, and still others that nitrogen fertilization decreased it. No reports on the effects of fertilizers on ascorbic acid in turnip greens were mentioned. In a recent publication, REDER *et al.* (**15**) found an effect of nitrogen fertilization on ascorbic acid content of turnips and a significant interaction between effects of nitrogen fertilization and of location where the plants were grown. They suggested that one of the reasons for the lack of agreement among investigators as to the effect of nitrogen on ascorbic acid content may be

because of this interaction and because differences associated with location are so much greater than the effect of fertilizer. This suggestion is in agreement with the results obtained here if one assumes that factors responsible for locational effects are the same as those that cause seasonal effects (i.e., climate variables), since an effect of nitrogen supply on ascorbic acid content (in sand cultures) was noted at one season of the year and not at another. REDER *et al.* found a consistent decrease in the ascorbic acid content of greens which received potassium fertilization, and they point out that this effect of potassium fertilization is not confirmed by the work of several other investigators with other plants. They did find a significant interaction of potassium effect and location indicating that potassium fertilization reduced the ascorbic acid content more sharply at one location than at others. In the work reported here, no significant effect of potassium fertilization of soils, nor of potassium supply to the plants in sand culture was noted with the exception that in sand cultures those treatments which were deficient in potassium produced plants which were low in ascorbic acid. This is in contrast to the above mentioned work of REDER *et al.* in that in our work a deficient supply of potassium resulted in a decrease in ascorbic acid instead of an increase.

A further comparison between the work presented here and that of REDER *et al.* seems worth while. In the work of the latter investigators, higher ascorbic acid values were obtained in the fall than in the spring, which is in contrast to our results. The marked effect of environmental factors other than soil variables is apparent in both series of investigations. REDER *et al.* found the lowest ascorbic acid values at the location with the highest average daily rainfall, and pointed out in comparing the average values at several locations, that there seemed to be an inverse relationship between the amount of rainfall and ascorbic acid content, and a direct relationship between the amount of sunshine and ascorbic acid. We have not been able to correlate the major variations of ascorbic acid which were obtained in this work with any particular environmental factor or factors. It seems apparent, however, that some factor or factors not under control or under observation in this work produced far greater effects on ascorbic acid content than were produced by any of the treatments used, with the possible exception of certain deficiency treatments in the sand cultures in which so little growth was produced that the plants doubtless would have had little commercial value as "greens."

MAYNARD and BEESON (**10**) state, "Studies that reveal any very useful data on the effect of fertilization or other cultural practices that might modify the carotene content of the plant are few because most of the reported findings are complicated by questions of varieties and as to the control of the many other factors of the plant's external environment." They discuss several investigations which indicate no effect of fertilization on the carotene content and a few which indicate some effect of fertilization. In their review, they cited some investigations which indicated an effect of

seasonal conditions and climatic environmental factors on carotene content. In the work reported here, little effect of fertilization was noted in plants growing in the soil. In sand cultures, there was fair agreement between the two experiments which indicated that deficiencies of sulfur, nitrogen, and potassium caused a decrease in carotene content, while a deficiency of phosphorus resulted in no decrease. Perhaps the most striking result of this work is the indication that any treatment which resulted in visible chlorosis resulted in appreciable decreases in carotene. This was further supported by the evidence of some correlation between chlorophyll and carotene content in the second experiment. No consistent correlation between the amount of growth and carotene content was found. The turnip plants used in the preliminary studies which were grown in the greenhouse during the winter time had much higher carotene values than any of those produced during the summer (the reverse relationship was found for ascorbic acid). There were differences in carotene content between the two experiments and differences between one location and another in each experiment indicating that environmental factors associated with climate are of considerable importance in determining carotene values.

It would appear that those climatic factors which lead to low ascorbic acid values tend to result in high carotene values since in the majority of the data, seasonal conditions and location factors correlated with high ascorbic acid values were also correlated with low carotene values and vice versa.

Since there was very little difference in the ascorbic acid or carotene values of the plants in one soil as compared with those in another soil when the soils were contained in pots, it seems unlikely that the locational differences noted in these experiments are due to soil conditions. That some of the variations might have been due to the particular date of harvest serves further to indicate the possibility that the environmental factors, such as light, temperature, humidity, etc., are of great importance in determining vitamin values. As a result of the work recorded here, a series of experiments with turnips has been carried out in chambers in which most of the environmental factors were under careful control. This work which is reported in another paper (**8**) has indicated that light intensity is of very great importance in determining vitamin content of turnip greens, and consideration of the data contained here certainly does not contradict such a conclusion. Variations such as occur between one location and another or between one harvest date and another might well be related to the light intensity prevailing just previous to harvest in each case. The significant conclusion that may be made from the experiments reported here would seem to be that variations in the supply of macronutrient elements in sand cultures and variations in fertilizer practices in soils seem to have little effect on the carotene and ascorbic acid content of the plants. Variations associated with location in which the plants are grown or season at which the plants are harvested seem to be of much greater magnitude.

Summary

1. Shogoin turnips were grown in sand cultures supplied with varying amounts of macronutrient elements to make a total of 87 treatments, in soil-pot-cultures, and soil field plots with 26 fertilizer treatments. Two complete experiments were conducted; one in the late spring and early summer, the other in the early fall.

2. At the time of harvest, ascorbic acid and carotene analyses were made. In addition, data were taken on the number of leaves, the total leaf length, fresh, and dry weights of roots and tops, length and diameter of the enlarged portion of the tap root, as well as detailed visual observations. The growth data were used as an indication of the responses of the plants to treatment, and an attempt was made to correlate these with the ascorbic acid and carotene values. The data for the first experiment were analyzed by statistical methods.

3. Preliminary analyses for ascorbic acid and carotene were made to determine the best methods of sampling and analyzing for these constituents.

4. With respect to the ascorbic acid content of turnip greens, both of these experiments indicate that the differences in environment between locations or between dates have a marked effect. Differences between the values obtained in the two experiments conducted at two different seasons were also relatively great. Only minor differences in ascorbic acid could be associated with variations in the supply of macronutrient elements to plants grown in sand culture except in complete deficiencies of some macronutrients. No differences were associated with fertilizer treatment in soil-pot-cultures or in field plots, in spite of the fact that variations in growth and appearance of the plants were relatively great in the different treatments.

5. The carotene content of the plants grown in the separate experiments varied greatly, indicating a marked influence of season. Variations in carotene content of plants grown in different locations in any given experiment were also great. No appreciable effect of fertilizer treatment on the carotene content of plants grown in soil was noted. In sand cultures, deficiencies of sulfur, nitrogen, and potassium caused a decrease in carotene content while a deficiency of phosphorus resulted in no decrease. Any treatment which resulted in visible chlorosis resulted in appreciable decreases in carotene. No consistent correlation between the amount of growth and the carotene content was found.

6. Both the ascorbic acid and carotene contents seem to be primarily influenced by environmental variables associated with season and location, and there was some evidence that those particular conditions which led to a high ascorbic acid content resulted in a low carotene content and vice versa.

7. A discussion is given of the possible significance of this work with the suggestion that light intensity may be the environmental factor playing the dominant rôle in determining ascorbic acid values.

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