EFFECT OF FERTILIZER AND ENVIRONMENT ON THE ASCORBIC ACID CONTENT OF TURNIP GREENS [1]

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INTRODUCTION

Numerous investigations have been conducted to determine the effect of fertilizer on the ascorbic acid content of vegetables. The methods of experimentation employed have varied widely, particularly with respect to the plants studied, the cultural methods used, and the composition of the fertilizers applied. In early work ascorbic acid was determined by biological methods and in later studies by chemical methods. The lack of agreement in the results obtained in these investigations may probably be attributed, in part, to dissimilarities in the experimental procedures employed. The results indicate, in general, that the ascorbic acid content of plants tends to increase as the plant yield is increased by fertilizer application, and that nitrogen and potassium are the fertilizer constituents most effective in increasing the formation of this vitamin.

The present study represents one phase of an investigation conducted cooperatively in six States to determine the cause of variations in the composition of vegetables produced by the same cultural methods in various sections of those States. The purpose of the study, in which three of the cooperating States participated, was to determine the effect of fertilizer treatment and environmental conditions on the ascorbic acid content of turnip (*Brassica rapa* L.) greens. The experiments were conducted at Norfolk and Blacksburg, Va.; Stillwater, Okla.; and Experiment, Ga.

MATERIALS AND METHODS

Four fertilizer factors were studied. In order that not only the individual effects of the fertilizers but also the possible interrelated effects might be determined, a factorial experiment was planned. A 2×2×2×2 factorial design consisting of 16 treatments with 2 replications at each location was used. The arrangement of treatments in each replication was randomized and different arrangements were used at each location. The treatments consisted of the application of nitrogen, phosphorus, potassium, and calcium in all possible combinations of high and low levels of nutrients; the check consisted of plants produced without fertilizer treatment, i. e., the zero level of application of all four nutrients. The 16 treatments were: NPKCa,

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NPK, NPCa, NP, NKCa, NK, NCa, N, PKCa, PK, PCa, P, KCa, K, Ca, and check.

Turnip seed of the Seven Top variety was supplied for the experiments by the United States Regional Vegetable Breeding Laboratory at Charleston, S. C. The seed was planted in rows 25 feet long and 30 inches apart. Each replicate consisted of 16 experimental rows with 2 border rows on each side of the area. The 2 replicates at each location were placed either side by side or end to end. Fertilizers were applied in a band 2 inches to the side of the row and 1 inch below the seed level. The fertilizers were applied at the following levels: 60 pounds per acre of nitrogen from ammonium sulphate, 60 pounds of P_2O_5 from superphosphate, 60 pounds of K_2O from muriate of potash, and 120 pounds of calcium from gypsum.

Records were kept of the maximum and minimum daily temperatures and the rainfall at each location.

Soil samples were taken after the ground was prepared for planting and before the fertilizers were applied. The samples were obtained in the following manner: A hole approximately 6 inches deep with a vertical side was dug with a garden trowel, and from the vertical side of the hole a thin, uniform slice of soil was taken from the surface to a depth of 6 inches. This operation was repeated 20 times in 2 diagonals across the field. The samples were thoroughly mixed and from the composite a sample was taken for analysis.

The pH values of the soils were determined by the use of glass electrodes. Rapid soil-test determinations for calcium, magnesium, nitrate nitrogen, and phosphorus were made by the perchlorate method of extraction devised by Dr. I. E. Miles[3]. Soil organic matter was determined by the method originally developed (*26*)[4] and later modified (*27*) by Schollenberger. Total exchange capacity and exchangeable calcium were determined by the methods of the Association of Official Agricultural Chemists (*1*).

When the first experiment was conducted at Norfolk, six samples of greens were analyzed from each experimental row. An examinaton of the results of this experiment by Immer's method (*12*) showed that there was need for a reduction in sampling error. This was achieved by increasing the number of samples to eight in the three experiments carried out in 1940. At Norfolk, Blacksburg, and Stillwater all rows of a single replicate and, in most cases, all rows of both replicates were sampled at the same time. At Experiment two collections of four samples were made on 2 different days after plants reached the proper size.

In view of the fact that the ascorbic acid content of plants varies thoroughout the day, samples were taken in the morning in each of the experiments, and all samples from a given location were collected at approximately the same time. At Norfolk and Blacksburg samples were taken from 8 to 8:30 a. m.; at Stillwater, from 7 to 7:30 a. m.; and at Experiment, at 7:30 and 9:30 a. m.

In sampling, large, medium, and small leaves were selected at random from average-sized plants. When the field was close to the laboratory, the samples were placed in a refrigerator within a few minutes after they were collected. At Stillwater, where the field was located some distance from the laboratory, the samples were

[3] Unpublished method.
[4] Italic numbers in parentheses refer to Literature Cited, p. 386.

placed in paper bags and packed at once with chipped ice in an insulated container in which they were conveyed to the laboratory.

In preparation for analysis, the leaves were thoroughly washed and rinsed in distilled water. Excess water was removed by patting them either with paper towels or cheesecloth. They were then placed in containers and stored in refrigerators at 2° to 4° C. until they were analyzed. All analyses were completed the same day on which the samples were collected. Duplicate determinations were made of the ascorbic acid content of samples from each row. Representative leaves of various sizes, selected for each sample, were cut or torn into strips which were well mixed. From them a 20-gm. sample was quickly weighed and placed in a glass mortar and covered with an acid mixture consisting of equal parts of 2 N H_2SO_4 and 0.25 N HPO_3.

At this point in the procedure there were some differences in the techniques employed. At Experiment, the method used for the determination of ascorbic acid was essentially that described by Bessey and King (*2*); in the other experiments, the method developed by Thornton (*29*) was used. In a comparision of the two methods, one of the investigators (M. S. Eheart), using both procedures to determine the ascorbic acid content of apples, found that the results obtained by the two methods were in close agreement. The same investigator found that the average percentage recovery of ascorbic acid from turnip greens was 96.9 percent for 25 samples.

The methods employed given briefly are as follows. At Experiment, the sample was ground under the acid mixture with 25 to 30 gm. of acid-washed sand, then transferred to 50-ml. centrifuge tubes. After centrifugation, the clear, supernatant liquid was poured off and the remaining material was again ground and centrifuged. This process was repeated a third time, the extracts were combined, and the volume was made up to 250 ml. with the acid mixture. Two 10-ml. aliquots of the well-mixed solution were titrated with a standardized solution of the dye 2, 6 dichlorophenolindophenol, which was added from a microburette. The dye solution, containing 60 mg. per 100 ml., was standardized daily against a solution of ascorbic acid containing 0.1 gm. Cebione (Merck) in 250 ml. of 3 percent HPO_3. The ascorbic acid solution was titrated against a 0.01 N iodine solution which was standardized against a 0.01 N As_2O_3 solution. One milliliter of 0.01 N iodine is equivalent to 0.88 mg. of ascorbic acid.

At the other laboratories, the procedure was as follows: The sample was finely ground under the acid mixture with 5 gm. of acid-washed sand. The mixture was transferred quantitatively to a 250-ml. volumetric flask and the volume was made up to 250 ml. with the acid mixture. Two portions of the well-mixed liquid were centrifuged for 1 minute, at 1,800 r. p. m. and a 5-ml. aliquot of the supernatant liquid from each portion was placed in a 125-ml. Erlenmeyer flask and titrated with the indophenol solution which was added from a microburette.

The indophenol solution was made up in Sörensen's solution, pH 7.0; 50 mg. of dye was used in 200 ml. of solution. At Norfolk and Blacksburg, the dye was standardized by the method used at Experiment except that the iodine solution was standardized against a standard solution of sodium thiosulfate. At Stillwater, a simplified method of standardization developed by Menaker and Guerrant (*17*) was used. In this method, an excess of potassium iodide is added to a measured volume of the indophenol solution in the presence of acid and the liberated iodine is titrated with a 0.01 N sodium thiosulfate solution, 1 ml. of which is equivalent to 0.88 mg. of pure ascorbic acid.

Analysis of variance of the data from each experiment was made in the usual manner. The effect of each fertilizer treatment was

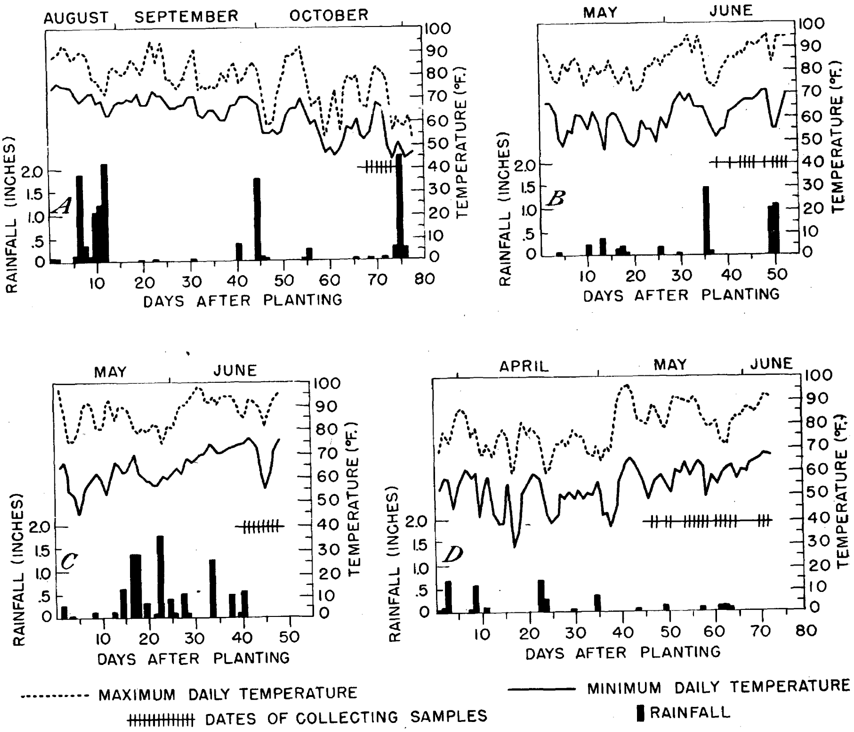

FIGURE 1.—Maximum and minimum daily temperatures, distribution of rainfall, and dates of planting and collecting turnip greens of the Seven Top variety for ascorbic acid determinations at Norfolk, Va., 1939 (*A*); Stillwater, Okla., 1940 (*B*); Blacksburg, Va., 1940 (*C*) and Experiment, Ga., 1940 (*D*).

determined by the method devised by Yates (*34*) for the analysis of factorial experiments. Chi-square tests showed the four experiments sufficiently homogeneous in error variances for satisfactory combination.

EXPERIMENTAL RESULTS

Data pertaining to the type and composition of the soil and meteorological conditions at each location during the growing season are presented in table 1. The dates of planting and collecting samples for analysis, maximum and minimum daily temperatures, and the distribution of rainfall during the growing season are shown graphically for each location in figure 1.

The average maximum temperatures at the four locations (table 1) ranged from 77.7° F. at Norfolk and Blacksburg to 83.7° at Stillwater, and the average minimum temperatures ranged from 54.2° at Blacksburg to 62.9° at Norfolk. Differences between the maximum and minimum daily temperatures (fig. 1) were smaller at Norfolk than at the other locations. The average daily rainfall (table 1) ranged from 0.06 inch at Experiment to 0.21 inch at Blacksburg. Distribution of rainfall (fig. 1) during the growing season varied considerably at the different locations. At Norfolk, 7.2 inches of rain fell during the first 13 days of the first one-third of the growing season; 2.43 inches in the second one-third; and 4.33 inches in the final period. The rains at Blacksburg were well scattered throughout the season, but the precipitation was highest during the second one-third of the period, when 6.17 inches of rain fell. At Stillwater, the rainfall was 1.26, 0.54, and 3.61 inches for the first, second, and final periods of the growing season, respectively. At Experiment, the rainfall was light and most of it fell during the first one-third of the season.

The results obtained in the 4 experiments are given in table 2, both separately and in combination. In each experiment, the average value given for the ascorbic acid content of greens receiving a given treatment is an average of the values obtained for all samples which received this treatment in the 2 replicates; in the experiment at Norfolk, this value is an average of the ascorbic acid content of 12 samples, 6 samples having been taken from a given treatment in each replicate; in the other 3 experiments, the value is an average of 16 samples.

TABLE 1.—*Type and composition of soils on which turnip greens (Seven Top) were produced, and meteorological data recorded during the growing season, at Norfolk and Blacksburg, Va.; Stillwater, Okla.; and Experiment, Ga.*

Location of experiment	Elevation	Soil type	Soil reaction	Composition of soil [1]							Meteorological data for growing period							
											Rainfall		Average temperature		Days—			
				Ca	Mg	N (nitrate)	P	Organic matter	Total exchange capacity [2]	Exchangeable Ca [2]	Total	Average daily	Maximum	Minimum	With rainfall over 0.01 inch	Clear	Partly cloudy	Cloudy
	Feet		*pH*					*Per cent*	*Milligrams*	*Milligrams*	*Inches*	*Inches*	°*F.*	°*F.*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Norfolk, Va	11	Norfolk sandy loam	5.8	L− [1]	L	M−	L+	2.47	7.6	2.3	13.08	0.17	77.7	62.9	27	32	33	35
Blacksburg, Va	2,170	Dunmore silt loam	5.5	T+	L−	M−	L	2.42	7.2	1.6	10.2	.21	77.7	54.2	52	27	29	44
Stillwater, Okla	880	Canadian loamy fine sand	6.0	H	M+	L+	L−	1.35	5.5	2.8	5.44	.10	83.7	59.6	25	50	29	21
Experiment, Ga	946	Cecil sandy clay loam	5.7	L	L−	M−	L+	1.62	6.3	3.8	4.01	.06	78.8	54.4	26	49	17	34

[1] Pounds per acre for letters used in recording the results of the buffered perchloric acid test.

Elements estimated	Trace (T)	Low (L)	Medium (M)	High (H)	Very high (VH)
Calcium	0–300	−1,000	−2,000	−3,000	−4,000
Magnesium	0–10	−30	−90	−150	−200
Nitrogen (nitrate)	0–5	−20	−50	−70	−100
Phosphorus	0–10	−50	−100	−250	−400

[2] Milligrams per 100 gm. of soil.

TABLE 2.—*The ascorbic acid content of turnip greens (Seven Top) grown in factorial design of 16 duplicate fertilizer treatments at 4 locations, and the effects [1] of the treatments at each location and for the 4 locations combined*

Fertilizer treatment	Plants grown at—									
	Norfolk, Va.		Blacksburg, Va.		Stillwater, Okla.		Experiment, Ga.		All locations	
	Ascorbic acid content [2]	Effect [3] of treatment	Ascorbic acid content	Effect of treatment	Ascorbic acid content	Effect of treatment	Ascorbic acid content	Effect of treatment	Ascorbic acid content [4]	Effect [5] of treatment
	Mg. per gm.	*Mg. per gm.*	*Mg. per gm.*	*Mg. per gm.*	*Mg. per gm.*	*Mg. per gm.*	*Mg. per gm.*	*Mg. per gm.*	*Mg. per gm.*	*Mg. per gm.*
NPKCa	2.2991	−0.0583	1.4062	−0.0093	1.4826	−0.0022	1.6194	−0.0105	1.6620	−0.0175
NPK	2.4723	−.0142	1.4013	+.0233	1.4938	−.0080	1.7769	+.0665	1.7403	+.0190
NPCa	2.3724	−.0569	1.3610	−.0126	1.5588	−.0034	1.9438	+.0634	1.7714	+.0013
NP	2.3528	−.0167	1.3934	+.0759**	1.5829	+.0017	1.8325	−.0577	1.7529	+.0020††
NKCa	2.5716	+.0268	1.2267	+.0006	1.5031	+.0166	1.7444	−.0626	1.7074	−.0067
NK	2.4281	+.0659	1.2232	+.0351	1.5468	−.0003	1.8506	−.0102	1.7178	+.0198
NCa	2.3683	−.0780*	1.2911	−.0062	1.5148	−.0257	2.0319	−.0362	1.7638	−.0337*
N	2.4873	+.0174	1.2761	+.0762**	1.5677	−.0944**	1.9950	−.1145*	1.7878	−.0292*††
PKCa	2.4129	−.0555	1.2230	+.0215	1.5982	+.0032	1.7813	−.0209	1.7099	−.0101
PK	2.2535	−.0103	1.1633	+.0193	1.5799	−.0252	1.8469	−.0535	1.6747	−.0179
PCa	2.5268	+.0124	1.2773	+.0011	1.6868	+.0187	2.0719	−.0576	1.8483	−.0076
P	2.3004	−.0730	1.2895	+.0603**	1.6239	−.0053	2.1613	−.0546	1.8133	−.0147††
KCa	2.3933	−.0094	1.1983	+.0058	1.5949	−.0111	2.0294	−.0404	1.7647	−.0141
K	2.3780	−.0183	1.2272	−.0512*	1.6316	−.0493*	1.8325	−.1927**	1.7266	−.0819**††
Ca	2.5206	+.0457	1.3061	+.0039	1.6598	−.0072	2.0594	+.0073	1.8442	+.0102
Check	2.4271		1.2842		1.6303		1.9275		1.7766	
Mean	2.4103		1.2842		1.5785		1.9065		1.7539	
Standard deviation	.2476		.1495		.1431		.3091		.2234	
Significant difference in average effects at the 5 percent level		.0762		.0398		.0381		.1054		.0288
Significant difference in average effects at the 1 percent level		.1053		.0551		.0527		.1458		.0384

F values for variance analysis at the 4 locations combined are: Places = 1,022.27**; treatments = 3.513**; treatment × places = 2.687**.
* Significant at the 1-percent level.
** Significant at the 5-percent level.
†† Interaction of treatment × places is significant at the 1-percent level.
[1] All data were used in calculating the effect of each fertilizer treatment. For example, the effect of nitrogen on the ascorbic acid content of plants grown at a given location was calculated in the following manner: After adding the replicates together to get a total for each of the 16 treatments the 8 containing nitrogen, i. e., N, NCa, NK, NKCa, NP, NPCa, NPK, and NPKCa, were added together and the 8 not containing nitrogen were added together. The difference between the positive combinations and the negative combinations is the total algebraic effect of nitrogen. (See table 3.) The effect of each of the single fertilizers at a given location was calculated in a similar manner. For the calculation of the effects of treatments containing two or more fertilizer constituents consult table 3 or Yates (*34*).
[2] Each value is an average of 16 samples from duplicate plots at all locations except at Norfolk, where the value is an average of 12 samples.
[3] Each value is an average of 128 samples from 16 plots at all locations except at Norfolk, where the value is an average of 96 samples.
[4] Each value is an average of 60 samples from 8 plots.
[5] Each value is an average of 480 samples from 64 plots.

The average effect of each fertilizer treatment was calculated for each experiment and these effects are shown in table 2. The method of calculation is shown in table 3. In all cases these effects make use of more of the data than do the averages compiled from the treatment alone; for example, in each experiment only 2 rows received nitrogen, but the effect of nitrogen in the experiment is calculated on the basis of 16 rows, since half of the 32 rows in the 2 replicates received nitrogen and half did not. At Norfolk, each effect was calculated from the ascorbic acid content of 96 samples; at the other locations, from the results of the analyses of 128 samples. The significance of these results was determined and significant effects are designated in the table.

As may be seen from an inspection of table 2, five of the fertilizer treatments gave significant effects at one or more places; these were the simple fertilizers, N, P, and K and the combinations NP and NCa. The largest number of significant effects in one experiment was observed at Blacksburg, where four treatments significantly affected the ascorbic acid content of the greens. Two treatments gave significant effects at Experiment, two at Stillwater, and one at Norfolk.

Treatment with nitrogen fertilizer gave the most interesting results; at Blacksburg and Norfolk, nitrogen produced an increase in ascorbic acid content (significant at Blacksburg), but at Stillwater and Experiment, the application of nitrogen resulted in significant decreases in this vitamin. The decrease was significant for the combination of the four places, and the interaction of N treatment × places was highly significant.

TABLE 3.—*Main effects and ineractions in a 4-factor experiment*

	Combination of treatments															
	Check	Ca	K	KCa	P	PCa	PK	PKCa	N	NCa	NK	NKCa	NP	NPCa	NPK	NPKCa
Total	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ca	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+
k	−	−	+	+	−	−	+	+	−	−	+	+	−	−	+	+
kca	+	−	−	+	+	−	−	+	+	−	−	+	+	−	−	+
p	−	−	−	−	+	+	+	+	−	−	−	−	+	+	+	+
pca	+	−	+	−	−	+	−	+	+	−	+	−	−	+	−	+
pk	+	+	−	−	−	−	+	+	+	+	−	−	−	−	+	+
pkca	−	+	+	−	+	−	−	+	−	+	+	−	+	−	−	+
n	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
nca	+	−	+	−	+	−	+	−	−	+	−	+	−	+	−	+
nk	+	+	−	−	+	+	−	−	−	−	+	+	−	−	+	+
nkca	−	+	+	−	−	+	+	−	+	−	−	+	+	−	−	+
np	+	+	+	+	−	−	−	−	−	−	−	−	+	+	+	+
npca	−	+	−	+	+	−	+	−	+	−	+	−	−	+	−	+
npk	−	−	+	+	+	+	−	−	+	+	−	−	−	−	+	+
npkaca	+	−	−	+	−	+	+	−	−	+	+	−	−	+	−	+

The effect of potassium in the fertilizer was to decrease the ascorbic acid in each of the four experiments. This decrease was significant in three places and for the combination of all places. The average decrease effected in ascorbic acid content was 8.2 mg. per 100 gm. of fresh material for 480 samples which were treated with fertilizer containing potassium, as contrasted with the same number of samples not fertilized with potassium. There was also a significant interaction between places and the treatment effect of potassium; this indicates that there was a much greater decrease due to potassium applications at Experiment than at any of the other three places.

Phosphorus gave a significant effect only at Blacksburg, where it increased the ascorbic acid content. At the other three places the effects were negative but not significantly so. The effect for P treatment × places was highly significant. Calcium was associated with an increase in ascorbic acid content in three places and with a decrease in one place, but none of the effects were significant. The interaction of Ca treatment × places was not significant.

The combination of NP gave a highly significant increase at Blacks burg, a nonsignificant increase at Stillwater, and nonsignificant decreases at the other two places. The interaction of NP treatment × places was highly significant. The combination of NK gave non-significant increases at two places and nonsignificant decreases at two places. The effect for the combination of four places was not significant. NCa gave decreases at four places, significant at one place, and the decrease for the combination of four places was significant.

The most striking observation to be made from the four experiments is that at Norfolk, where the maximum vitamin formation was obtained, the mean ascorbic acid content of the greens (2.4103 mg. per gm.) was nearly twice as great as that of greens grown at Blacksburg, where the mean ascorbic acid content (1.2842 mg. per gm.) was the lowest observed in the experiments. The mean ascorbic acid content of the greens produced at Experiment and Stillwater was 1.9065 and 1.5785 mg. per gm., respectively. The influence of places was 13.75 times as great as the most important average effect, i. e., the effect for potassium, but when compared to the effect of potassium at Experiment, place difference was only five times as great.

DISCUSSION

Several earlier investigators (*16*, *21*, *23*, *24*, *25*, *32*, *33*) found no changes in the ascorbic acid content of various plants as a result of fertilizer treatment. A few workers have reported that certain fertilizer ingredients altered the ascorbic acid content of some plants. None of these investigators, however, worked with turnip greens. The significant decreases in ascorbic acid produced by nitrogen in the fertilizer at two places and the significant decrease obtained by combining the results from all four places are directly opposed to the findings of a number of other workers who found nitrogen to increase the ascorbic acid content of many plants (*3*, *6*, *10*, *11*, *13*, *19*). The highly significant interaction of N treatment × places in the present experiments suggests one reason for the lack of agreement; i. e., the effect of place is more important than the effect of fertilizer.

The most consistent effect obtained in the four experiments was the decrease in the ascorbic acid content of greens which received potassium fertilizer. The opposite effect was observed by Hester (*7*), Ijdo (*10*), and others (*11*, *22*); Isgur and Fellers (*13*) and Fellers et al. (*4*) found no change in the ascorbic acid of plants as a result of potassium fertilization.

The highly significant interaction of potassium effect × places, although all places showed a decrease in ascorbic acid content, indicates that potassium reduced the ascorbic acid content more sharply in one location than in another. The greatest reduction was found at Experiment, Ga.

The effect of phosphorus in the fertilizer was not significant for the combination of the four places. This confirms the results of previous studies (*10*, *13*) with spinach and Swiss chard. Ott (*19*), however, increased the amount of this vitamin in potatoes by applying nitrogen and phosphorus, and Pfützer and Pfaff (*22*) increased it in vegetables by the addition of phosphate to a phosphorus-deficient soil. The ineffectiveness of calcium in the fertilizer in significantly modifying the ascorbic acid content of the greens in the present experiments confirms the results obtained by Ijdo (*10*) with spinach.

NCa was the only fertilizer combination to give an effect significant for the combination of the four places. Several investigators (*3*, *5*, *9*, *28*, *31*) have found that the ascorbic acid of different vegetables was increased by the application of the fertilizer combinations NPK or NPKCa, but the effects of these combinations in the present experiments were not significant. Ott (*20*) found that tomatoes grown without fertilization contained more vitamin C than those fertilized with NPK.

The results show that under the conditions imposed in these experiments, fertilizer treatment significantly affected the ascorbic acid content of turnip greens, but that the effect of place was more important than the greatest effect obtained with any fertilizer. Factors contributing to the effect of place are the type and composition of the soil and meteorological conditions during the growing season. Different types of soil were used at the four locations (table 1), but the results of the soil analyses (table 1) do not reveal very marked differences in soil composition. It is possible that trace elements in the soil exert an effect on ascorbic acid formation; Hester (*8*), for example, indicated that the amount of manganese in the soil is an important factor affecting the ascorbic acid content of plants. From the results obtained, no conclusions may be drawn as to the relationship between either soil composition or temperature and the ascorbic acid content of turnip greens.

The fact that the greens which had the highest ascorbic acid content were grown in the fall suggests a seasonal variation in the ascorbic acid content of turnip greens. No conclusion may be drawn from this fact, but the observation is made in view of the results obtained by Tressler, Mack, and King (*30*), who found that spinach produced at two locations in the fall contained one-third more ascorbic acid than spinach grown in the spring at the same locations. Because of the seasonal effect observed by these investigators, the three spring crops are compared; of these, the crop having the highest ascorbic acid content (1.9065 mg. per gm.) was grown at Experiment, where the average daily rainfall was lower than at any of the other three places, and 49 percent of the days were clear. At Blacksburg, where the greens had the lowest average ascorbic acid content (1.2842 mg. per gm.), the average daily rainfall was greatest and only 27 percent of the days of the growing season were clear. These results indicate an inverse relationship between the amount of rainfall and ascorbic acid content of the greens and a direct relationship between the amount of sunshine and the formation of ascorbic acid. Several investigators (*14*, *15*, *18*) have shown that the ascorbic acid content of plants is directly influenced by light intensity. Experiments are in progress to investigate further the effect of season and other environmental factors on the ascorbic acid content of turnip greens.

SUMMARY AND CONCLUSIONS

Experiments were conducted at Norfolk and Blacksburg, Va., Stillwater, Okla., and Experiment, Ga., to determine the effects of fertilizer treatment and environmental conditions on the ascorbic acid content of turnip greens. A factorial design was used for applications of N, P, K, and Ca in all possible combinations at a high and low level for each nutrient.

Uniform methods of planting and fertilizing were used with seed of the variety Seven Top from a single source. Meteorological data were recorded for each experiment and soil samples from each area were analyzed for calcium, magnesium, nitrate nitrogen, phosphorus, organic matter, total exchange capacity, exchangeable calcium, and pH values. Chemical methods were used for the determination of ascorbic acid. The results of the experiments were analyzed statistically, both separately and in combination, and are discussed in terms of the calculated effects of the fertilizer treatments.

Three of the single fertilizer treatments, N, P, and K, and two of the fertilizer combinations gave significant effects at one or more places. Nitrogen fertilizer gave increases in ascorbic acid at two places (significant at one place) and significant decreases at two places. The decrease for the four places combined was significant. The interaction of N treatment × places was highly significant.

The most consistent results were obtained with potassium fertilizer, which produced a decrease in ascorbic acid in each experiment; the decreases were significant at three places and highly significant for the combination of places. Many investigators working with other plants have found the application of potassium fertilizers to increase the ascorbic acid content.

Phosphorus gave a significant increase at one place and nonsignificant decreases at three places. The effect for P treatment × places was highly significant. Calcium produced no significant effects and the interaction of Ca treatment × places was not significant.

The combination of NP gave a highly significant increase at one place and the interaction of NP treatment × places was highly significant. NK gave nonsignificant increases at two places, and nonsignificant decreases at two places. The effect was not significant for the combination of places. NCa gave decreases at all places, significant at one place, and the decrease was significant for the combination of four places.

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 (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. These variations did not appear to be directly related to differences in soil composition or to differences in temperature. Fertilizers represent at most only a small part of the total environment of a plant; consequently it could not be expected that controlled applications of fertilizer would have as much effect as the total environment involving differences in soil and weather.

Influence of season is suggested by the fact that the one fall crop had the highest ascorbic acid content, but this result is confounded with the effect of place so that no definite conclusion may be reached. The highest ascorbic acid content of the three spring crops (1.9065 mg. per gm.) was found in greens which were produced at the place having the lowest average daily rainfall and where 49 percent of the days in the growing season were clear; the lowest ascorbic acid content (1.2842 mg. per gm.), was found in greens which received the greatest average daily rainfall and the least amount of sunshine. 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.

LITERATURE CITED

(1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1940. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Ed. 5, 757 pp., illus. Washington, D. C.

(2) BESSEY, O. A., and KING, C. G.
1933. THE DISTRIBUTION OF VITAMIN C IN PLANT AND ANIMAL TISSUES, AND ITS DETERMINATION. Jour. Biol. Chem. 103: 687–698.

(3) BURRELL, R. C., BROWN, H. D., and EBRIGHT, V. R.
1940. ASCORBIC ACID CONTENT OF CABBAGE AS INFLUENCED BY VARIETY, SEASON, AND SOIL FERTILITY. Food Res. 5: 247–252.

(4) FELLERS, C. R., YOUNG, R. E., ISHAM, P. D., and CLAGUE, J. A.
1934. EFFECT OF FERTILIZATION, FREEZING, COOKING AND CANNING ON THE VITAMIN C AND A CONTENT OF ASPARAGUS. Amer. Soc. Hort. Sci. Proc. (sup. v., 1934) 31: 145–151, illus.

(5) HAHN, F.-V., and GÖRBING, J.
1933. VITAMINSTUDIEN. SECHSTE REIHE. EINFLUSS DER DÜNGUNG AUF DEN C-VITAMINGEHALT VON SPINAT. Ztschr. f. Untersuch. der Lebensmitl. 65: [601]–616, illus.

(6) HAUSEN, S. VON.
1933. ZUR KENNTNIS DER VITAMINBILDUNG IN PFLANZEN. [VITAMIN FORMATION IN PLANTS.] Acta Chem. Fennica (B) 6: 62. (Abstract in Chem. Abs. 28: 2388. 1934.)

(7) HESTER, J. B.
1940. THE INFLUENCE OF POTASH UPON THE YIELD AND QUALITY OF TOMATOES. Amer. Fert. 93 (11): [5]–8, 24, 26, illus.

(8) ———
1941. MANGANESE AND VITAMIN C. Science 93: 401.

(9) HOFFMAN, I. C., KRAUSS, W. E., and WASHBURN, R. G.
1938. THE EFFECT OF SOME VARIETAL, CULTURAL, HARVESTING, AND STORAGE CONDITIONS UPON THE CONTENT OF CERTAIN MINERAL SALTS AND VITAMINS IN TOMATO FRUITS. Ohio Agr. Expt. Sta. Ann. Rpt. 56 (Bul. 592): 66–67.

(10) IJDO, J. B. H.
1936. THE INFLUENCE OF FERTILIZERS ON THE CAROTENE AND VITAMIN C CONTENT OF PLANTS. Biochem. Jour. 30: 2307–2312.

(11) ILLI͡UVIEV, V. P. [ILLYUVIEV, V. P.], and ULANOVA, M. N.
1937. VITAMIN C CONTENT IN BERRIES AND VEGETABLES DEPENDING ON VARIETY AND CULTIVATION. Bul. Appl. Bot., Genet., and Plant Breeding, Sup. 84, Vitamin Prob. 2: 95–107. [In Russian. English summary, p. 5, summaries section.] (Abstract in Chem. Abs. 33: 1405. 1939.)

(12) IMMER, F. R.
1932. A STUDY OF SAMPLING TECHNIC WITH SUGAR BEETS. Jour. Agr. Res. 44: 633–647.

(13) ISGUR, B., and FELLERS, C. R.
1937. A PRELIMINARY STUDY OF THE RELATIONSHIP BETWEEN VITAMIN C CONTENT AND INCREASED GROWTH RESULTING FROM FERTILIZER APPLICATIONS. Amer. Soc. Agron. Jour. 29: 890–893.

(14) KOHMAN, E. F., and PORTER, D. R.
1940. SOLAR RAYS AND VITAMIN C. Science 92: 561.

(15) McIntosh, J.
1938. SOME FACTORS AFFECTING THE VITAMIN C CONTENT OF TOMATOES AND RUTABAGAS. Maine Agr. Expt. Sta. Ann. Rpt. 1937–38. (Bul. 391): 320–321.
(16) Mack, G. L., Tapley, W. T., and King, C. G.
1939. VITAMIN C IN VEGETABLES. X. SNAP BEANS. Food Res. 4: 309–316.
(17) Menaker, M. H., and Guerrant, N. B.
1938. STANDARDIZATION OF 2,6-DICHLOROPHENOLINDOPHENOL: AN IMPROVED METHOD. Indus. and Engin. Chem., Analyt. Ed., 10: 25–26, illus.
(18) Mituda, H.
1938. STUDIES ON VITAMIN C. 2. ON THE VARIATIONS OF THE VITAMIN C CONTENT IN SOME FRUITS. Agr. Chem. Soc. Japan Jour. 14: 1335–1340, illus. (Abstract in Nutr. Abs. and Rev. 8: 973. 1939.)
(19) Ott, M.
1937. ÜBER DEN GEHALT VON FELD- UND GARTENFRÜCHTEN AN VITAMIN C UND CAROTIN BEI VERSCHIEDENER DÜNGUNG. Angew. Chem. 50: 75–77, illus. (Abstract in Nutr. Abs. and Rev. 7: 45. 1937.)
(20) ———
1938. PFLANZENQUALITÄT, VOLKSERNÄHRUNG UND DÜNGUNG. Forschungsdienst 5: 546–552, illus. (Abstract in Nutr. Abs. and Rev. 8: 401. 1938.)
(21) Pfaff, C., and Pfützer, G.
1937. ÜBER DEN EINFLUSS DER ERNÄHRUNG AUF DEN CAROTIN- UND ASCORBINSÄUREGEHALT VERSCHIEDENER GEMÜSE- UND FUTTERPFLANZEN. Angew. Chem. 50: 179–184. (Abstract in Chem. Abs. 31: 3620. 1937.)
(22) Pfützer, G., and Pfaff, C.
1938. ZUSAMMENHÄNGE ZWISCHEN MINERALSTOFFAUFNAHME UND VITAMINGEHALTEN VON PFLANZEN [INTERDEPENDENCE OF MINERAL UPTAKE AND VITAMIN CONTENT OF PLANTS.] Fortschr. der Landw.- Chem. Forsch. 1937: 104–115, illus. (Abstract in Chem. Abs. 33: 3954. 1939.)
(23) Scheunert, A.
1938. ÜBER ERNÄHRUNGSVERSUCHE MIT VERSCHIEDEN GEDÜNGTEN GEMÜSEN. ÜBER DEN VITAMINGEHALT VON GEMÜSEN, WELCHE EINERSEITS MIT STALLDUNG, ANDERSEITS MIT STALLDUNG+NPK GEDÜNGT WURDEN. [NUTRITION INVESTIGATIONS WITH VARIOUS FERTILIZED VEGETABLES. THE VITAMIN CONTENT OF VEGETABLES FERTILIZED WITH MANURE AND WITH MANURE PLUS N, P AND K MIXTURE.] Ernährung 3: 67–69. (Abstract in Chem. Abs. 32: 3846. 1938.)
(24) ——— Reschke, J.
1938. ÜBER DEN VITAMIN C-GEHALT VON GEMÜSEN, WELCHE EINERSEITS MIT STALLDUNG, ANDERERSEITS MIT STALLDUNG+NPK GEDÜNGT WORDEN WAREN. Forschungsdienst 6: 34–48.
(25) ——— Reschke, J., and Kohlemann, E.
1940. ÜBER DEN VITAMIN C-GEHALT DER KARTOFFELN. IV. MITTEILUNG: ÜBER DEN EINFLUSS VERSCHIEDENER DÜNGUNG. Biochem. Ztschr. 305: 1–3.
(26) Schollenberger, C. J.
1927. A RAPID APPROXIMATE METHOD FOR DETERMINING SOIL ORGANIC MATTER. Soil Sci. 24: 65–68, illus.
(27) ———
1931. DETERMINATION OF SOIL ORGANIC MATTER. Soil Sci. 31: 483–486.
(28) Sugawara, T.
1938. INFLUENCE OF FERTILIZERS ON THE ASCORBIC ACID (VITAMIN C) CONTENT OF SPINACH. Jour. Sci. Soil and Manure, Japan 12: 567–576. (Abstract in Chem. Abs. 33: 1077. 1939.)
(29) Thornton, N. C.
1938. EXTRACTION AND DETERMINATION OF VITAMIN C IN PLANT TISSUE. Boyce Thompson Inst. Contrib. 9: 273–281.
(30) Tressler, D. K., Mack, G. L., and King, C. G.
1936. VITAMIN C CONTENT OF VEGETABLES. I. SPINACH. Food Res. 1: 3–7.

(31) VOGEL, F.
1939. ERGEBNISSE DER ZWEIJÄHRIGEN GEMEINSCHAFTSARBEITEN ÜBER DEN EINFLUSS DER DÜNGUNG AUF ERTRAG UND GÜTE VON WEISSKOHL. Bodenk. u. Pflanzenernähr. 15: 134–139.
(32) WACHHOLDER, K., and NEHRING, K.
1938. ÜBER DEN VITAMIN C-GEHALT VERSCHIEDENER KARTOFFELSORTEN UND SEINE ABHÄNGIGKEIT VON DER DÜNGUNG. Bodenk. u. Pflanzenernähr. (n. s.) 9–10: 708–724, illus.
(33) WENDT, H., PIES, [R.], PRÜFER, J., and others.
1938. ÜBER ERNÄHRUNGSVERSUCHE MIT VERSCHIEDEN GEDÜNGTEN GEMÜSEN. Ernahrung 3: [53]–69.
(34) YATES, F.
1937. THE DESIGN AND ANALYSIS OF FACTORIAL EXPERIMENTS. Imp. Bur. Soil Sci. Tech. Commun. 35, 95 pp.

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