

VITAMIN CONTENT OF SEVEN TOP TURNIP-GREEN PLANTS AS RELATED TO DRY
MATTER AND THE DISTRIBUTION OF THESE CONSTITUENTS IN THE PLANT

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INTRODUCTION

The importance of leafy, green vegetables as a source of vitamins for human consumption is well established. For several years an extensive study has been underway to determine the effect of soil and climatic conditions on the composition of one such leafy, green vegetable, turnip greens. This study, Southern Regional Project S-5, is being conducted by the Southern Cooperative Group in Georgia, North Carolina, Oklahoma, Texas and Porto Rico; at the Oklahoma Agricultural Experiment Station the work is being carried out under the supervision of Dr. Ruth Reder.

The original project was designed to study variations in the composition of the leaf blades of the plants, but in 1950 a sub-project was initiated at the Oklahoma Station to determine the composition of all plant parts. The purpose of this phase of the project was to determine the total amounts of ascorbic acid, thiamine, riboflavin, and carotene, in the whole plant, to relate such components to plant dry matter as the plants matured, and to determine the percentage distribution of the vitamins and dry matter among the various plant parts.

REVIEW OF LITERATURE

Dry Matter It is generally agreed that the accumulation of dry matter in plants is determined by all the factors involved in photosynthesis. These factors include the chlorophyll content and possibly, the carotene content of the plant, temperature, oxygen and carbon dioxide supply, light intensity, light quality, availability of moisture and minerals, carbohydrate accumulation and the activity of the plants enzymes.

Blackman and Wilson (6) have shown that the net assimilation rate, or dry matter accumulation, over a range of one-tenth to full daylight, is linearly related to the logarithm of the light intensity, and that the light intensity at which the growth rate is maximum varies between plant species. Hunter, Kelly and Somers (22) have found that plants grown on soils having high moisture tensions and low moisture content, have a higher percentage of dry matter than those grown on soils with low moisture tension and high moisture content, but that higher yields are associated with low moisture tension. This report has been confirmed by Janes (24). Other factors, such as heavy fertilization (23) and excessive amounts of nitrogen or phosphorous (44), have been found to decrease the dry matter content of plants; turnip roots are especially susceptible to excessive amounts of phosphorous.

Ascorbic Acid Factors affecting the ascorbic acid content of plants have been more thoroughly studied than those affecting any of the other known vitamins. Light intensity and quality have been found to affect the ascorbic acid content of all plants that have been studied (12, 11, 1, 10, 30, 41, 18, 38, 21). The effect of light quality has been demonstrated in illumination experiments with filters. Mei, Hsieh, and Chen (30) found that orange light was most effective in promoting an increase in ascorbic acid in etiolated soybean seedlings, and Heller (21) reported that sunlight was more favorable than ultra violet light or light filtered through glass for ascorbic acid formation in wheat seedlings. He also found that ascorbic acid synthesis was accelerated by increased intensities of light. Gallup and Reder (13), in agreement with the work of Heller (21), found that a considerable amount of vitamin C was produced in sprouted seeds during germination in the dark.

Aberg (1) found that in full grown tomato leaves ascorbic acid content was proportional to light intensity in the wave length range below 700 millimicrons up to 80 mg. cal/sq. cm. At light intensities below 50 mg. cal/sq. cm. ascorbic acid content decreased with age, while above this intensity it increased. The leaves of tomato plants placed in darkness after a period of illumination lost ascorbic acid rapidly. Aberg concluded that ascorbic acid may be synthesized independently of light, as in sprouting seeds, as well as by direct photosynthesis. Visible light of short wave length seems to have no

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value in ascorbic acid synthesis. Hamner and Parks (18) believe that light intensities one week before harvest are the dominant factor in determining the ascorbic acid level in plants.

The effect of temperature on the ascorbic acid content of different plant species has been studied by several workers. Aberg (1) found that plants grown at 15.5°C . contained about 30 percent more ascorbic acid than those grown at 23.0°C ., and other workers have also reported that low temperatures favor formation of ascorbic acid (11). Lecat (26) reported that a rise in temperature increased ascorbic acid formation but that its destruction was increased to a greater extent, so that lower concentration of the vitamin resulted. Geographic factors, which affect weather conditions influence the ascorbic acid content of plants (11); greater amounts of vitamin C have been found in the same species as habitats extended from low to high altitudes (45).

The moisture content of the soil also affects the ascorbic content of plants; low soil moisture has been shown to favor a high ascorbic acid content, fresh weight basis. In an experiment by Hunter et. al. (22) high soil moisture content lowered the ascorbic acid content of turnip green plants on a fresh weight basis (22).

The concentration of ascorbic acid varies in the different plant parts. Venkataramani (40) found that leaves and rapidly growing tissues contain approximately 80 percent of the total vitamin content of the plant, while the stems and roots have a low ascorbic acid content. Lecat (26) also reports that the leaf blades of plants contain more ascorbic acid than the conduction tissue. Demers (10) found that in potato stems there is

a definite longitudinal ascorbic acid gradient, as well as a chlorophyll gradient; the amounts of these constituents decrease from the base to the apex. This gradient was the same whether expressed on a fresh or dry basis, since the moisture gradient is negligible in stems over 20 cm. in length.

Karl G. Hamner (17) has stated, "Variations in the ascorbic acid content of plants such as might be encountered under field conditions are influenced so markedly by differences between varieties and by climatic conditions that the possible influence of soil conditions and fertilizer practices probably have little practical importance". Wynd (45) is of the opinion that the dry matter yield of both oats and rye is more important in governing the yields of protein, carotene and ascorbic acid than is the concentration of these substances in the tissue. Janes (23), in a study of the effect of geographical location and fertilizer level on the variations in dry weight, carotene and ascorbic acid content of certain vegetables, concluded that there was little or no effect of fertilizer level on the percentage of these constituents in the plants; high ascorbic acid content was correlated with high dry weight. Reder et. al. (35) found a highly significant positive correlation between rainfall and the ascorbic acid content of turnip greens on both the wet and dry basis.

Despite the fact that the effects of climate tend to obscure the effect of fertilizer on the ascorbic acid content of plants, small effects due to fertilizer treatment (40) have been observed. For example, the application of potassium fertilizers has been found to increase ascorbic acid content in turnip greens and other plants (8). Reder et. al. (34), however, found that fertilization

with potassium produced a decrease in the ascorbic acid content of field grown turnip greens. Some workers (40, 27) have found that under certain conditions phosphorous and nitrogen fertilization increases the ascorbic acid content of plants, but others (35) have reported that nitrogen fertilization did not significantly affect the ascorbic acid content of turnip greens. Thus, on the basis of reported work, it appears that the ascorbic acid content of plants cannot be varied with any degree of certainty by addition of the common fertilizers.

There appears to be an interrelation between the effects of the plant concentration of magnesium and nitrogen and the concentration of ascorbic acid. It has been reported that ascorbic acid is synthesized from a plant sugar (14); any factor which would affect sugar formation, photosynthesis, would presumably affect the concentration of ascorbic acid. Photosynthesis has been found to vary inversely with the carbon-nitrogen ratio in the plant (15), and since in oat leaves a low concentration of magnesium was accompanied by low nitrogen and a high concentration of ascorbic acid (42), apparently a high concentration of ascorbic acid is associated with a low rate of photosynthesis. Noggle et. al. (32) found that this observation is supported by the fact that there was a significant negative partial correlation between the sugar fraction and ascorbic acid concentration in oat plants when the magnesium relation to sugar was held constant.

Riboflavin Watson and Noggle (42) found that growth in terms of dry substance was significantly correlated with riboflavin concentration in immature oat plants. The correlation coefficients relating the growth of the leaves and stems, dry weight per plant,

with riboflavin concentration in mcg. per gram were 0.743 and 0.771, respectively; these correlations were significant at the five percent level. The correlation of the growth of roots with riboflavin concentration was not significant, 0.593. The authors suggest that this relationship may indicate a direct dependence of the growth of plant tissue upon the synthesis of an adequate amount of riboflavin. Gustafson (16) reports that riboflavin was more abundant in plants grown in the temperature range of 28 to 30°C. than in those grown in the 5 to 10°C. range, and that summer grown plants therefore should contain more riboflavin than those grown in the rainy winter season (7). Some workers believe that riboflavin is more concentrated in the mature leaves of the young plant (43) others believe the opposite to be true (7).

There was a high positive correlation between nitrogen and riboflavin in the leaves of the oat plants when a nitrate deficiency produced the lowest riboflavin concentration in leaf, stem and roots (42). Other workers have reported that the nitrogen content and dry weight of tomato plants had no particular correlation with the riboflavin content (43). In some experiments all mineral deficient treatments caused a lower riboflavin concentration in the leaves and stems where most of the riboflavin is found (41). Toxic concentration of manganese produced a 35 percent increase in riboflavin in turnips (29). The apparent effect of magnesium on riboflavin seems to be a nitrogen effect since leaves low in magnesium are low in nitrogen (42).

Thiamine Factors which influence the thiamine content of plants are not as well known as those affecting other plant vitamins.

Gustafson (16) found that thiamine was more abundant in certain vegetables and legumes grown in the 28 to 30°C. temperature range than in plants grown in the 5 to 10°C. temperature range. Withner (43) reports that thiamine seems to occur in higher concentrations in the leaf tip region. Some experiments show that the nitrogen and dry weight content of tomato plants have no particular correlation with the amount of thiamine present. In minor element studies boron at a toxic concentration increased the thiamine content of turnip greens about 60 percent (29).

Carotene Carotene has been suggested as a possible photosynthetic factor. The work on this provitamin is not as complete or extensive as that on ascorbic acid but certain factors, such as soil moisture, season, and fertilization have been found to affect the carotene content of plants. It has been found that plants grown on soils having a high moisture tension have a higher carotene content on the fresh basis, than plants grown on soils of low moisture tensions (22). Fernandez (12) found that vegetables grown in Porto Rico increased in carotene content during the winter and, in general, decreased during the summer. Bernstein, Hamner and Parks (5) report a marked influence of season on the carotene content of turnip greens.

Janes (23) found that heavy fertilizer application increased the carotene concentration of certain plants. In general, carotene content is associated with the greenness of a plant; a dark green color is related to high carotene (8). Several workers (5, 17, 8) have found that any soil treatment which causes visible chlorosis results in appreciable decreases in carotene

content. In controlled experiments, Lucas (23) found that copper increased the carotene content of greenhouse grown spinach, barley, carrots and oats. Sulfur, nitrogen and potassium caused a decrease in carotene content and phosphorous caused no decrease (5). In grasses the correlation of variations in carotene and protein content was highly significant (25), but no consistent correlation was found between growth and carotene content (5).

For all the vitamins of plants studied to date the highest yield of vitamin per acre was obtained with the fertilizer treatments which gave the highest yield per acre. Hansen (19) reports that hereditary rather than environmental factors appear to be the most important in determining the ascorbic acid content of vegetables.

MATERIALS AND METHODS

Production of Crops

Three experiments were conducted with Seven Top turnip greens, Brassica rapa L., grown at Stillwater in the fall of 1950 and at Stillwater and Perkins in the spring of 1951. In each experiment the seed was planted in 12 100-foot rows two and one-half feet apart and a complete fertilizer was applied as a side dressing to each row when the field was planted. To facilitate sampling, each row was divided into ten-foot segments and starting at the end of the rows, the first segments of the rows were numbered one through six from left to right, then the second segments were numbered seven through 12 from right to left; this system was followed until all the 120 segments were numbered. Duplicate samples were taken at each harvest and each sample consisted of an equal number of plants harvested from each of five segments which were randomly assigned except that each was from a different one-fifth of the field. All segments were sampled from the same end and plants were taken in order of their occurrence. A minimum of ten plants were used for each sample; these were always the first two guarded plants in each segment. If more than ten plants were required, more than two plants were taken from each segment.

Preparation of Samples

Sampling was begun about five weeks after planting and was continued for six weeks; samples were collected at 8 A. M. on

two fixed days each week. At harvest, the plants were pulled or dug from the row, shaken to remove loose soil from the roots, and placed in paper bags in which they were taken at once to the laboratory where the roots were removed and the leaves separated. The leaves and roots were washed in tap water, rinsed twice in distilled water and allowed to drain. The samples were placed in the refrigerator until the next operation; this short cooling period allowed the leaves to regain their turgidity. In the next operation the mid-ribs were carefully removed from the leaves and the fresh weights of leaf blades, midribs and roots determined. Except when the samples were small, the two halves of the leaf blades were put in separate composites, only one of which was used for analysis. The remaining composite was dried in a forced draft oven at 70°C. and the dry weight determined. This weight was used in calculating the total leaf blade dry weight. The leaf blades, midribs and roots were diced and each composite mixed separately and then analyzed for moisture, ascorbic acid, carotene, thiamine and riboflavin. Carotene was not determined in the roots.

Analytical Procedures

The chemical methods used in this experiment were those employed by the Southern Cooperative Group in the Soils-Weather project (39).

Moisture Moisture content of plant parts was determined by drying duplicate 50-gram samples of the fresh diced material to constant weight in a forced draft oven at 70°C.

Ascorbic Acid The Heinze-Kanapaux method was used in the determination of ascorbic acid in the fresh material (20). Ten grams

of the diced sample was placed in a Waring blender with 200 ml. of one percent meta-phosphoric acid, blended for three minutes, and then filtered. One ml. of the clear extract was placed in an Evelyn colorimeter tube with ten ml. of dilute 2, 6-dichlorophenol-indophenol dye solution (17 mg. per liter) and the percent transmission of the partially reduced dye solution was determined immediately. The reading was taken in an Evelyn colorimeter fitted with a number 520 filter and set at 100 percent transmission with a tube containing the same solution as the sample tube, but with sufficient ascorbic acid added completely to decolorize the dye.

Readings of the unreduced dye were obtained by mixing one ml. of one percent meta-phosphoric acid solution with ten ml. of the dye and reading the transmission of this solution after the instrument had been set at 100 percent transmission with a tube containing the completely decolorized dye. The logarithm of the mean reading of the unreduced dye was subtracted from the logarithm of the mean sample reading, and the amount of ascorbic acid present in the extract was read from a prepared standard curve in which differences of the logarithm of the numbers were plotted against ascorbic acid concentration. In the calculation of the total amount of vitamin in the extract the total volume of extract was determined by taking the sum of the volumes of extract and the amount of moisture in the sample.

Riboflavin Riboflavin was determined by the method outlined by Peterson (33). This analysis was conducted in a darkened room. An extract was made of the plant material with 0.04 N. sulfuric acid and used for the thiamine and riboflavin determinations.

This extract was obtained by grinding 10 to 20 grams of material with 40 ml. of the acid in a semimicro blender for five minutes. The extract was transferred to a 250 ml. Erlenmeyer flask, covered with a piece of moisture proof cellophane, and autoclaved for 15 minutes at 15 lbs. pressure per sq. in. To the cooled extract was then added ten ml. of 0.5 percent Takadistase in a sodium acetate-acetic acid buffer with a pH of 4.5. The extract was incubated at 40°C. overnight, diluted to 250 ml. in volume, filtered, and stored in the refrigerator until analyzed. A five ml. aliquot of the extract of the sample was placed in each of two tubes; to the first tube was added five ml. of 0.4 percent acetic acid solution, and to the second, an equal volume of acetic acid containing the internal standard, 1.20 mcg. of riboflavin. To each tube was first added one ml. of one percent potassium permanganate, then exactly two minutes later, one ml. of three percent hydrogen peroxide. The tubes were stoppered, shaken for 30 seconds then decanted into the photofluorometer cuvettes. The purpose of this treatment was to oxidize fluorescent materials other than riboflavin to non-fluorescent substances and this increased the accuracy of the determination. Before reading the samples the Model 12 Coleman Electronic photofluorometer was set at 100 with a dilute sodium fluorescein solution. The blank reading, caused by the fluorescence of substances other than riboflavin, was taken for each sample following the reduction of the riboflavin present by the addition of a small amount of solid sodium hydrosulfite. The internal standard was used to determine the amount of riboflavin equivalent to one scale division, and this constant was

multiplied by the corrected sample reading to determine the riboflavin content of the extract.

Thiamine Thiamine was determined by an adaptation of the method of Conner and Straub (9). Thiamine concentration in the extract was determined by oxidizing the thiamine to thiochrome and measuring the fluorescence of this compound with the Model 12 Coleman Electronic photoflurometer. This analysis was conducted in a darkened room. Duplicate field samples were analyzed as a unit with an internal standard added to only one of the two samples. A 25 ml. aliquot of the acid extract of one sample was placed on each of two columns of activated Decalso which removed the thiamine from the solution but allowed all other soluble material, including riboflavin, to pass through. To one of the columns 2.0 mcg. of thiamine was added as an internal standard. Twenty-five ml. of the second sample of the pair was added to a third column. The columns were washed three times with hot water and the washings discarded. Elution of the thiamine was effected with 30 to 40 ml. of hot acid 25 percent potassium chloride solution. The eluate was transferred to a 50 ml. volumetric flask, made to volume and stored in the refrigerator until analyzed.

Before analysis the chromatographed thiamine solutions were warmed to room temperature. Four reaction vessels were used in the oxidation of each pair of samples. To three of the vessels there was added three ml. of the oxidizing reagent, 0.03 percent potassium ferric cyanide in 15 percent sodium hydroxide solution. To the fourth vessel the blank, there was added three ml. of 15 percent sodium hydroxide solution. Five ml. of the first sample

the first sample plus internal standard and the second sample were added to the first, second and third tube respectively; five ml. of sample one was added to the blank. Fifteen ml. of redistilled isobutyl alcohol was added to each of the four tubes which were then shaken vigorously for 60 seconds. After the aqueous and alcohol phases had separated the aqueous phase was removed with a siphon and the isobutyl layer containing the thiochrome was dried with non-fluorescent anhydrous sodium sulfate. The alcoholic solutions were decanted into a photoflurometer cuvettes and the four tubes were read at the same time following the reaction. The photoflurometer was set at 50 percent scale deflection with a dilute solution of quinine sulfate. The thiamine content of an extract was calculated by using the constant calculated from the difference between the readings of the sample plus standard and sample. To calculate the thiamine concentration of the extract, this constant, the amount of thiamine per scale division deflection, was multiplied by the reading of the sample minus the reading of the blank.

Carotene Carotene was determined by the method developed by Moore and Ely (31). Five grams of leaf blades or ten grams of midribs were placed in a blender with 60 ml. of Skellysolve B and 90 ml. of 95 percent ethyl alcohol. While the blend was mixing, enough 95 percent ethyl alcohol was added to give a foaming mixture. The fresh material was ground for five minutes, then filtered into a 500 ml. separatory funnel. Separation of the alcohol and petroleum ether phases was effected by the addition of distilled water. The water alcohol layer was washed

with petroleum ether and the washings added to the ether extract which was then thoroughly washed with distilled water and dried over anhydrous sodium sulfate. After the volume of the extract was determined, an aliquot was concentrated to a volume of about 25 ml. This aliquot was run through a chromatographic column consisting of two parts activated di-calcium phosphate and one part Dyno; the column removed all plant pigments except carotene. The eluate was made to 100 ml. with Skellysolve B, mixed, and the transmission read at 440 millimicrons in the Evelyn colorimeter with the instrument set at 100 percent transmission with Skellysolve B. The concentration of carotene was then determined by referring to a standard carotene curve, in which the log of the percent transmission was plotted against carotene concentration.

Statistical Analysis

The mean weight of the plants was determined by taking the sum of the mean weights per plant, of leaf blades, midribs and roots. The vitamin content of the whole plant was calculated as the sum of the amounts of vitamin present in each of the plant parts.

Statistical analysis (36) of the data from the three experiments included: analysis of variance of the dry matter, ascorbic acid, riboflavin, thiamine and carotene content per plant, the amount of vitamin per gram dry matter in the whole plant, and the percent distribution of dry matter and vitamin in the various parts of the plant; calculation of correlation coefficients for plant weight and vitamin content and for the amount per plant of the four vitamins; and finally, a regression study of plant vitamin content on plant weight.

RESULTS AND DISCUSSION

Vitamin Content as Related to Dry Weight

The pattern of behavior of plant constituents was found to be remarkably consistent in the three experiments. Table I presents mean plant dry weight and the mean total amounts of ascorbic acid, riboflavin, thiamine, and carotene in plants taken in 12 successive harvests in each of the experiments.

From the data presented in table I it is evident that in each experiment total vitamin content tended to vary directly with dry weight. As the dry weight of the plant increased with maturity the total amount of ascorbic acid, riboflavin, thiamine and carotene also increased. Maximum plant weight and maximum vitamin content occurred at the twelfth harvest in the first and third experiments, and at the eleventh harvest in the spring experiment at Stillwater. In the latter experiment there was a sharp decrease both in plant dry weight and vitamin content at the twelfth harvest. Maximum plant weight and vitamin content attained were less in the third experiment at Perkins than in either of the experiments at Stillwater, and were greater in the spring experiment (second) than in the fall experiment (first) at the latter location. The mean dry weights of plants from all harvests in the first, second and third experiments were 24.03 gm., 27.03 gm., and 17.84 gm., respectively.

The mean amounts, per plant, of dry matter, ascorbic acid, riboflavin, thiamine and carotene at each harvest for the three

combined experiments are presented in table II. Increases in plant weight and vitamin content over a period of six weeks were large; the mean maximum dry weight was 17.5 times the dry weight of the plants at the first harvest, and the amounts of ascorbic acid, riboflavin, thiamine and riboflavin increased 13.6, 10.7, 14.0 and 8.8 fold respectively.

The result of the analysis of variance for the three experiments is presented in table III. Variation among experiments was highly significant for all constituents; the mean dry weight of plants and the amount of each of the vitamins per plant were significantly higher in the first and second experiments conducted at Stillwater than in the third experiment at Perkins. Differences between the spring and fall experiments at Stillwater were not significant; this is of interest since growing conditions during these two seasons were markedly different. Differences in dry matter and vitamin content of plants at the successive harvest were highly significant. It is of interest to note that the mean total amount of ascorbic acid per plant was about 1000 times the mean amount of thiamine and about 500 times the mean amount of riboflavin. The riboflavin per plant was consistently about twice the thiamine content per plant. The numerical values of these inter-vitamin relationships were consistent in all experiments.

Since the amount of vitamin per plant tended to vary with total weight, correlation coefficients were calculated to determine the reality and significance of this relationship. As shown in table IV, positive correlation coefficients for plant dry weight and the amounts, per plant, of ascorbic acid, thiamine,

riboflavin and carotene were highly significant in all experiments. This relationship was unaffected either by season or location; correlation coefficients were equally high in plants produced in different seasons at the same location and at different locations in the same season.

Correlation coefficients were determined for the fresh weight of plant and the amount of the four vitamins per plant, in the second and third experiments. These coefficients, which are shown in table IV, were also positive and highly significant.

To determine whether correlation between plant dry weight and vitamin content was the same at different stages of maturity, partial correlation coefficients were determined with age of plant held constant. The partial correlations (table IV) were significant, but they were appreciably lower than the total correlation. This suggested the possibility that the concentration of vitamin in the plant changes as the plant matures.

To determine whether such changes had occurred, the amount of each vitamin per gram dry weight of whole plant was calculated for all samples in each experiment; mean values for the three experiments combined are presented graphically in figure 1. In this figure it is evident that as the plants matured there was a marked and progressive decrease in the amount of carotene and riboflavin per gram dry weight of whole plant tissue; over a period of six weeks carotene content decreased from 0.43 mg. to 0.19 mg. per gm., and riboflavin from 25.9 mcg. to 13.8 mcg. per gm. The results of the analysis of variance presented in table V shows that the variation among harvests was highly significant for both

carotene and riboflavin. There was also a highly significant variation among harvests for the amount of ascorbic acid per gram dry plant weight (table V); this decrease in concentration was not as marked as was observed in the case of carotene and riboflavin, nor was it continuous from an early to a late stage of growth. Figure 1 shows a difference between the level of ascorbic acid at the first five harvests and the vitamin level at the last seven harvests. Variations from 9.92 to 10.4 mg. and from 7.82 to 8.82 mg. ascorbic acid per gram dry plant weight, were observed at the first and second levels of vitamin content, respectively. There was no significant variation among harvests in the amount of thiamine per gram dry plant weight. Differences in the amounts of ascorbic acid, riboflavin and carotene per gram dry weight of plant with maturity, explain, at least in part, the lessened correlation between plant weight and vitamin content when age of the plant was held constant.

Regression coefficients, table VI, were calculated for the amount of vitamin per plant on plant dry weight in all experiments and on plant fresh weight in the second and third experiments. An analysis of errors of estimate showed that there was no significant variation among the regression coefficients in the three experiments for ascorbic acid, thiamine and carotene. There was a significant variation among the regression coefficients for riboflavin; the regression coefficient in the third experiment differed from that in the other two.

On the assumption of a linear relationship, equations were derived in each experiment for the estimation of the amount, per

plant, of ascorbic acid, riboflavin, thiamine, and carotene from the dry weight of the plant. In the second and third experiments equations were also derived for the estimation of these vitamins from the fresh weight of the plant. These equations are shown in table VI.

Since the analyses of errors of estimate showed that there was no significant variation among experiments in the regression coefficients for ascorbic acid, thiamine, and carotene, an equation for the estimation of the amount of each of these vitamins per plant was derived from the three experiments combined. Data from the first and second experiments were used for the calculation of an equation for the estimation of riboflavin. These equations and the values from which they were derived are shown graphically in figures II, III, IV and V. It is of interest to note that in each of the figures the line representing the combined equation follows very closely the general trend of the actual values plotted.

A study of the correlation of the plant content of each of the four vitamins with each other showed a highly significant correlation for each pair of vitamins in each of the experiments.

In general it might be said that the yields of dry matter are more important in determining the total yields of vitamin than variations in the content of the plant tissue. Other workers are in agreement with this concept (34, 36).

Distribution of Plant Constituents

One of the more important aspects of this problem was to determine the distribution of dry matter and vitamins among the

leaf blades, midribs and roots. The mean percentage distribution of dry matter and vitamins at each harvest are presented in table VII for the three experiments combined; the data are shown graphically in Figure VI. Significant variations, as determined by an analysis of variance of the three experiments, are shown in table IX.

As the plants matured, the percentage of dry matter in the leaf blades decreased significantly from about three-fourths to one-half of the total plant dry matter. There were also significant decreases, with maturity, in the percentage of ascorbic acid and riboflavin in the leaf blades; the decrease in the leaf blade content of ascorbic acid, from about 70 percent to 57 percent of the whole plant content, closely approximated the decrease in the percentage of dry matter in the blades, but the decrease in the percentage of riboflavin from about 90 to 80 percent was considerably less than the decrease in the percentage dry matter in this plant part. There were no significant changes with maturity in the percentages of carotene and thiamine in the leaf blades; carotene remained nearly constant at about 95 percent throughout the six weeks' growing period, and although the percentage of thiamine decreased about ten percent, the change was not significant.

The dry matter of the midribs increased significantly from about 23 to 39 percent of the whole plant as the plant matured. The percentage of ascorbic acid and riboflavin in the midribs also increased, but the increases were not so great as the change in dry matter and were not significant; the percentage of ascorbic acid increased from 20 to 32 percent of the total amount present,

and the percentage of riboflavin increased from 9 to 14 percent of the total plant content. The percentage of thiamine in the midribs varied between 6 to 13 percent, with no consistent pattern of variation; the percentage of carotene remained nearly constant at three to six percent during the successive harvests.

In the roots, the percentage of dry matter increased significantly from about five to 16 percent of the total plant dry matter; small but significant increases also occurred in the percentages of ascorbic acid, riboflavin and thiamine.

The mean percentage distribution of dry matter, ascorbic acid, riboflavin, thiamine and carotene in each of the three experiments is presented in table VIII. As is indicated in table IX there were significant differences among experiments in the percentage of total dry matter, ascorbic acid, riboflavin and thiamine in the leaf blades. An examination of the data in table VIII will show that the mean percentage of dry matter in the leaf blades was significantly greater in the fall experiment than in the two experiments conducted in the spring. Variations among experiments in the percentage of ascorbic acid, riboflavin and thiamine in the leaf blades were significant only between the fall and spring experiments (table VIII) at Stillwater and were apparently the result of the higher percentage of dry matter in the leaf blades in the former experiment. The mean percentage of total dry matter in the midribs was somewhat greater in the spring experiment at Stillwater than in the other two experiments (table VII), and as a result the percentages of ascorbic acid, riboflavin and thiamine in the midribs in this experiment were significantly high

er than in the other two.

There was no significant variation among experiment in the percentage of dry matter in the roots, but there were significant differences in the percentages of ascorbic acid, riboflavin and thiamine; the percentages of these vitamins in the roots in the fall experiment were less than in the two spring experiments.

The mean overall percentages of distribution of dry matter and vitamins among the plant parts are shown in table X. As may be seen in this table, the distribution of ascorbic acid followed that of dry matter more closely than did the other vitamins; about 65.5 percent of the total plant ascorbic acid was located in the leaf blades, as compared to 54.5 percent of total plant dry matter, and the percentages of this vitamin in the other plant parts were slightly less than the percentage of dry matter. The proportions of the other vitamins in the leaf blades were much greater than the proportion of total plant dry matter; about 84 percent of the plant content of riboflavin and thiamine, and about 95 percent of the total plant carotene content were located in the leaf blades. There was no carotene and only a small percentage of riboflavin and thiamine in the roots; there was somewhat more riboflavin than thiamine in the midribs, and only a trace of carotene.

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TABLE I

Mean amounts, per plant, of dry matter, ascorbic acid, riboflavin, thiamine and carotene in Seven Top turnip greens over a period of six weeks.

Harvest	Mean ¹ amount of constituent per plant				
	Dry matter	Ascorbic acid	Riboflavin	Thiamine	Carotene
First experiment: Stillwater, Fall 1950					
	Gm.	Mg.	Mcg.	Mcg.	Mg.
1	3.53	35.75	89.1	25.2	1.58
2	3.59	35.95	99.3	32.5	1.77
3	7.83	73.35	185.4	80.4	2.66
4	8.88	86.97	204.8	77.7	3.65
5	12.93	144.09	292.3	127.6	5.02
6	20.46	190.71	390.5	175.6	7.49
7	24.61	233.35	469.9	191.9	8.74
8	32.48	291.17	504.7	288.5	10.17
9	43.18	396.86	735.7	321.8	11.46
10	43.36	370.67	661.7	374.1	8.90
11	42.89	344.49	740.3	391.3	7.17
12	45.71	375.63	820.9	409.7	9.72
Second experiment: Stillwater, Spring, 1951					
1	1.99	20.5	50.5	21.4	0.76
2	2.81	27.9	71.2	32.9	1.11
3	8.02	93.0	148.4	92.9	2.87
4	10.68	119.6	203.4	105.7	3.23
5	14.33	139.8	252.9	122.7	4.35
6	28.33	189.9	502.6	226.7	7.16
7	27.12	238.1	492.4	209.1	7.28
8	35.74	313.0	673.2	408.5	9.04
9	41.89	349.6	759.9	309.6	9.10
10	50.68	478.3	749.4	409.1	12.92
11	60.55	424.9	875.2	512.4	13.76
12	42.29	350.3	546.6	309.5	7.40
Third experiment: Perkins, Spring, 1951					
1	2.45	23.0	66.4	38.4	0.63
2	2.63	25.9	64.3	24.5	1.08
3	7.73	74.2	183.7	61.2	2.65
4	8.41	87.0	160.8	59.3	2.97
5	14.11	127.8	265.2	111.5	4.79
6	17.52	147.1	296.4	116.1	4.91
7	11.01	92.7	178.5	77.1	2.77
8	24.81	198.9	352.9	221.6	5.89
9	24.69	230.8	386.1	180.7	5.43
10	24.13	214.2	399.6	235.6	6.78
11	36.60	310.3	588.6	281.0	7.70
12	40.08	321.8	434.6	259.9	6.85

¹Mean of duplicate samples.

Analysis of Variance for the constituents in turnip-
green plants at 12 harvests, in three experiments

Mean total amounts of dry matter, ascorbic acid, riboflavin, thiamine and carotene in Seven Top turnip-green plants in three experiments during a six weeks' growing season.

Mean ¹ amount of constituent per plant					
Harvest	Dry matter	Ascorbic acid	Riboflavin	Thiamine	Carotene
	Gm.	Mg.	Mcg.	Mcg.	Mg.
1	2.66	26.4	68.7	28.3	1.09
2	3.01	29.9	78.2	30.0	1.32
3	7.86	80.1	172.5	78.2	2.73
4	9.32	97.8	189.7	80.9	3.26
5	13.79	137.2	270.1	120.6	3.22
6	22.10	175.9	396.5	172.8	6.52
7	20.91	188.0	380.3	159.3	6.27
8	30.84	267.7	510.2	306.2	8.37
9	36.60	325.7	627.3	270.7	8.66
10	39.89	354.4	603.6	339.6	9.53
11	46.68	359.9	734.7	394.9	9.54
12	42.69	349.2	600.7	353.4	7.99

¹Each value is the mean of values obtained in the analysis of duplicate samples from each of three experiments.

L. S. D. for harvests	9.32	76.22	167.24	76.31	2.94
L. S. D. for experiments	4.66	38.11	83.62	38.15	1.48

TABLE III
Analysis of variance for total amounts of constituents in turnip-
green plants at 12 harvests, in three experiments

Constituent		Dry matter	Ascorbic acid	Riboflavin	Thiamine	Carotene
Source of Variation	D.F.	Mean squares				
Experiments	2	527.0482**	37,231.95**	197,777.87**	60,746.01**	37.05**
Harvests	11	1,516.5868**	98,497.92**	317,341.50**	105,279.62**	58.66**
Experiments X Harvests (Experimental error)	22	60.5570	4,051.34	19,506.70	4,061.23	6.04
Error (Sampling Error)	36	32.6518	2,440.84	9,923.88	2,536.38	2.79
		Gm.	Mg.	Meg.	Meg.	Mg.
Mean		22.97	199.37	386.02	194.56	5.83
Standard deviation		7.78	63.65	139.66	63.72	2.46
Coef. of variation, %		33.87	31.93	36.18	32.75	42.16
L. S. D. for harvests		9.32	76.22	167.24	76.31	2.94
L. S. D. for experiments		4.66	38.11	83.62	38.15	1.48

TABLE IV

Total and partial* correlation coefficients for plant dry weight and the total amounts of ascorbic acid, riboflavin, thiamine and carotene in Seven Top turnip-green plants in three experiments.

Experiment		Ascorbic acid		Riboflavin		Thiamine		Carotene	
Number	Description	Total	Partial	Total	Partial	Total	Partial	Total	Partial
1	Stillwater, fall	0.992	0.914	0.990	0.894	0.989	0.874	0.886	0.598
2	Stillwater, spring	0.963	0.787	0.970	0.888	0.966	0.815	0.962	0.906
3	Perkins, spring	0.990	0.919	0.946	0.673	0.963	0.715	0.930	0.590

Total correlation coefficients for plant fresh weight and the total amount of ascorbic acid, riboflavin, thiamine and carotene.

2	Stillwater spring	0.955	0.950	0.922	0.907
3	Perkins, spring	0.984	0.969	0.965	0.946

*Partial correlation coefficients for the amount of vitamin per plant and plant dry weight with age of plant held constant.

TABLE V

Analysis of variance for the amount of vitamin per gram
dry matter of whole plant at 12 harvest in
three experiments

Constituent		Ascorbic acid	Riboflavin	Thiamine	Carotene
Source of variance	D. F.	Mean squares			
Experiments	2	1.035	31.650*	3.415	0.01610**
Harvests	11	5.000**	92.210**	7.629	0.03536**
Experiments X Harvests (Experimental error)	22	1.132	6.522	5.627	0.00233
Error (Sampling error)	36	0.294	1.469	0.5511	0.000464
		Mg.	Mcg.	Mcg.	Mg.
Mean		9.13	19.0	8.8	0.301
Standard deviation		1.06	2.55	2.37	0.048
Coef. of variation, %		11.66	13.42	26.83	16.05
L. S. D. for harvests		1.27	3.06	—	0.058
L. S. D. for experiments		0.64	1.42	—	0.058

TABLE VI

Equations for estimation of the total amounts of ascorbic acid, riboflavin, thiamine, and carotene in Seven Top turnip-green plants from fresh and dry weight.

Experiment No. Description	Equations for estimation of total amounts of ascorbic acid, riboflavin, thiamine, and carotene in turnip-green plants from plant dry matter content. (X_1)			
	Ascorbic acid (X_2)	Riboflavin (X_3)	Thiamine (X_4)	R Carotene (X_5)
1 Stillwater, fall	$\hat{X}_2 = 16.866 + 8.242 X_1$	$\hat{X}_3 = 57.529 + 15.620 X_1$	$\hat{X}_4 = 4.672 + 8.461 X_1$	$\hat{X}_5 = 2.034 + 0.187 X_1$
2 Stillwater, spring	$\hat{X}_2 = 23.495 + 7.592 X_1$	$\hat{X}_3 = 50.424 + 14.553 X_1$	$\hat{X}_4 = 14.180 + 8.236 X_1$	$\hat{X}_5 = 0.726 + 0.217 X_1$
3 Perkins, spring	$\hat{X}_2 = 8.065 + 8.208 X_1$	$\hat{X}_3 = 62.793 + 12.253 X_1$	$\hat{X}_4 = 10.787 + 7.181 X_1$	$\hat{X}_5 = 1.261 + 0.175 X_1$
Equations for the estimation of total amounts of ascorbic acid, riboflavin, thiamine, and carotene in turnip-green plants from the plant fresh weight (X_0)				
2 Stillwater, spring	$\hat{X}_2 = 29.235 + 0.659 X_0$	$\hat{X}_3 = 66.374 + 1.246 X_0$	$\hat{X}_4 = 28.472 + 0.688 X_0$	$\hat{X}_5 = 11.58 + 0.0179 X_0$
3 Perkins, spring	$\hat{X}_2 = -4.972 + 0.863 X_0$	$\hat{X}_3 = 36.166 + 1.327 X_0$	$\hat{X}_4 = -1.770 + 0.716 X_0$	$\hat{X}_5 = 0.905 + 0.0189 X_0$

TABLE VII

Mean percentage distribution of dry matter, ascorbic acid, riboflavin, thiamine, and carotene in Seven Top turnip-green plants at 12 harvests in three combined experiments.

Harvest	Dry matter	Ascorbic acid	Riboflavin	Thiamine	Carotene
Leaf blades					
1	72.33	72.75	88.72	90.55	95.45
2	72.44	73.87	88.40	91.22	96.35
3	62.97	67.58	83.63	79.82	95.85
4	61.49	68.93	84.28	87.97	95.10
5	60.98	67.03	85.63	79.25	96.22
6	54.46	63.27	82.55	81.10	95.97
7	57.84	65.20	84.15	88.52	96.40
8	55.71	63.23	82.50	86.00	96.35
9	53.06	62.78	81.05	79.28	95.90
10	53.89	64.30	82.26	82.20	97.08
11	51.11	59.25	78.93	77.70	95.77
12	45.48	57.57	78.32	81.85	93.93
Midribs					
1	23.00	20.82	9.23	5.18	4.55
2	21.46	19.25	9.15	5.97	3.65
3	26.51	22.80	12.85	11.83	4.17
4	28.37	23.13	12.13	6.07	4.90
5	27.62	23.50	10.30	12.77	3.78
6	35.44	27.58	14.35	12.93	4.05
7	31.67	27.28	12.67	5.72	3.60
8	29.30	26.03	11.75	6.75	3.65
9	32.14	26.38	12.90	11.43	4.12
10	31.79	26.08	12.13	8.12	2.92
11	34.14	30.93	15.08	11.88	4.25
12	38.77	32.00	14.03	8.07	6.07
Roots					
1	4.67	6.43	2.07	4.27	0.00
2	6.09	6.88	2.41	2.83	0.00
3	10.53	9.65	3.50	8.33	0.00
4	10.15	7.95	3.55	6.00	0.00
5	11.40	9.45	4.00	7.93	0.00
6	10.10	9.20	3.01	6.00	0.00
7	10.49	7.52	3.15	5.77	0.00
8	15.00	10.72	5.77	7.23	0.00
9	14.80	10.87	6.07	9.30	0.00
10	14.32	9.62	5.63	9.68	0.00
11	14.75	9.82	6.00	10.40	0.00
12	15.75	10.42	7.67	10.12	0.00

TABLE VIII

Mean percentage distribution of dry matter, ascorbic acid, riboflavin, thiamine and carotene among the leaf blades, midribs and roots of Seven Top turnip-green plants in each of the experiments.

Experiment	Mean ¹ percentage of total amount of constituent per plant				
	Dry Matter	Ascorbic Acid	Riboflavin	Thiamine	Carotene
	<u>In Leaf blades</u>				
Stillwater, fall	61.45	69.30	85.93	87.27	95.79
Stillwater, spring	56.66	61.86	80.75	79.83	95.91
Perkins, spring	57.33	65.30	83.43	84.26	95.89
L.S.D. for experiments	3.70	4.56	2.89	4.54	--
L.S.D. for harvests	7.40	9.12	5.78	9.08	--
Stillwater, fall	28.42	23.06	11.03	6.95	4.21
Stillwater, spring	31.67	28.30	14.00	12.36	4.09
Perkins, spring	29.96	25.12	11.63	7.37	4.11
L.S.D. for experiments	--	3.79	2.53	3.61	--
L.S.D. for harvest	6.41	7.58	5.06	7.22	--
Stillwater, fall	10.14	7.65	3.04	5.78	--
Stillwater, spring	11.67	9.84	5.24	7.80	--
Perkins, spring	12.70	9.67	4.94	8.38	--
L.S.D. for experiments	--	1.10	1.10	2.77	--
L.S.D. for harvests	--	2.20	2.20	5.54	--

¹Mean of 24 samples; duplicate samples taken at 12 harvests.

TABLE IX

Significance of variation among experiments and harvests in the percentage distribution of dry matter and vitamin content of Seven Top turnip greens at 12 harvests in three experiments.

Plant part	<u>Dry matter</u>		<u>Ascorbic acid</u>		<u>Riboflavin</u>		<u>Thiamine</u>		<u>Carotene</u>	
	E ¹	H ²	E	H	E	H	E	H	E	H
Leaf blades	*	**	*	*	**	*	**	N.S.	N.S.	N.S.
Midribs	N.S.	**	*	N.S.	*	N.S.	**	N.S.	N.S.	N.S.
Roots	N.S.	**	**	**	**	**	*	*	—	—

¹Variation among experiments

²Variation among harvests

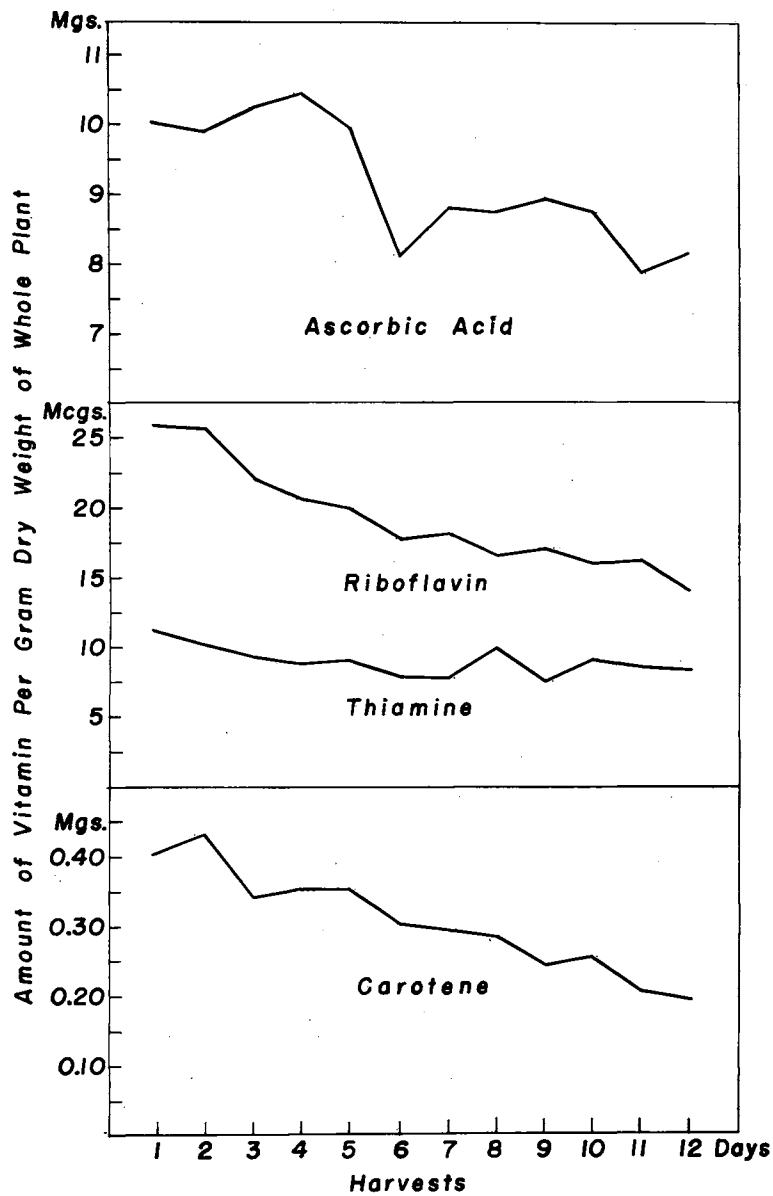
TABLE X

Mean percentage distribution of dry matter, ascorbic acid, riboflavin, thiamine, and carotene among the leaf blades, midribs and roots of Seven Top turnip-green plants in all experiments.

Plant part	Mean ¹ percentage of total amount of constituent per plant				
	Dry matter	Ascorbic acid	Riboflavin	Thiamine	Carotene
Leaf blades	54.48±6.18	65.49±7.62	83.37±4.83	83.79±7.59	95.86±1.95
Midribs	30.02±5.35	25.49±6.32	12.22±4.23	8.89±6.02	4.14±1.72
Roots	11.50±3.78	9.02±1.83	4.41±1.84	7.32±3.54	— —

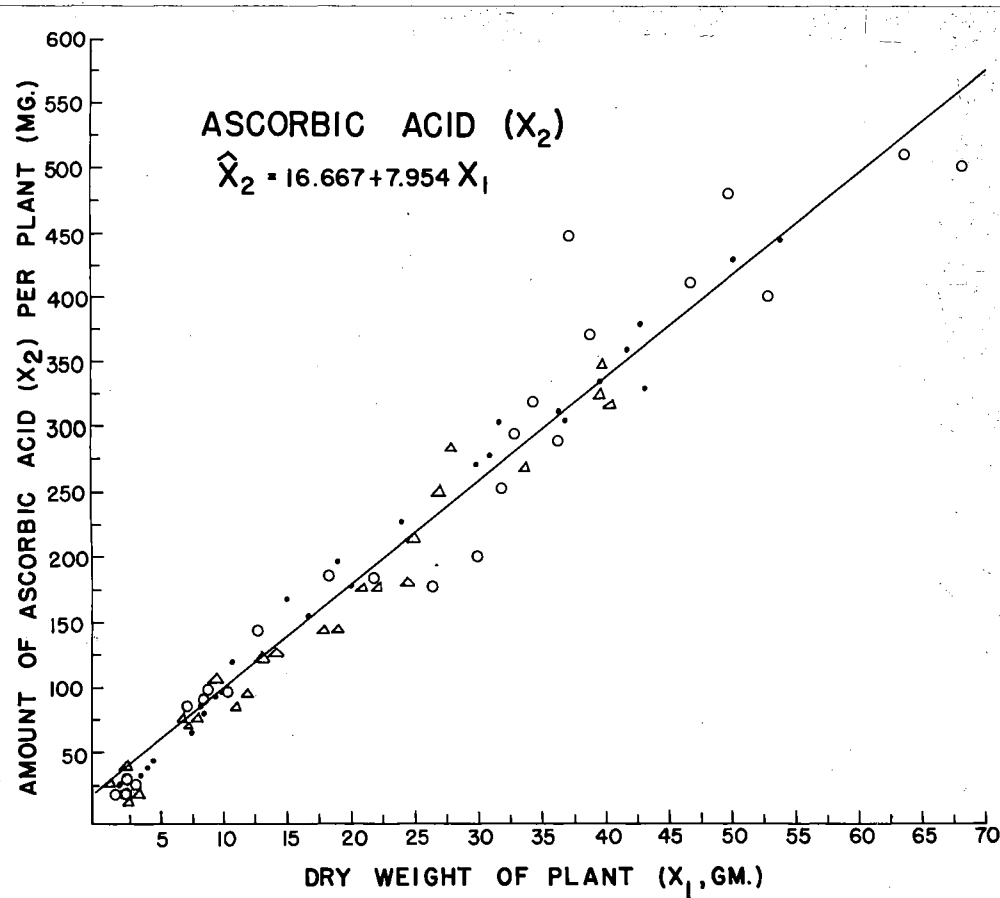
¹Mean of 72 samples; duplicate samples taken at 12 harvests in each of three experiments.

FIGURE I



Mean amount of ascorbic acid, riboflavin, thiamine and carotene per gram dry weight of whole plant at 12 harvests in three experiments combined.

FIGURE II



Regression of total amount of ascorbic acid per plant on dry plant weight in Seven Top turnip greens in three experiments.

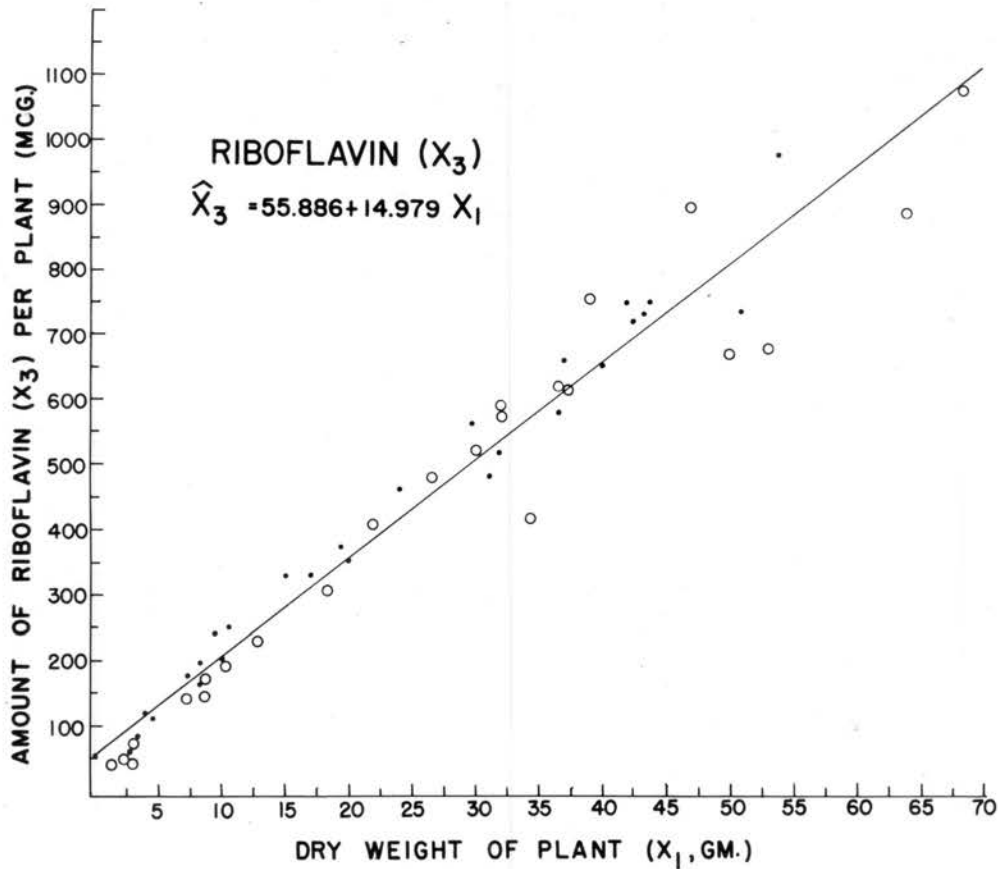
Stillwater, fall 1950 = Δ

Stillwater, spring 1951 = \bullet

Perkins, spring 1951 = \circ

FIGURE III

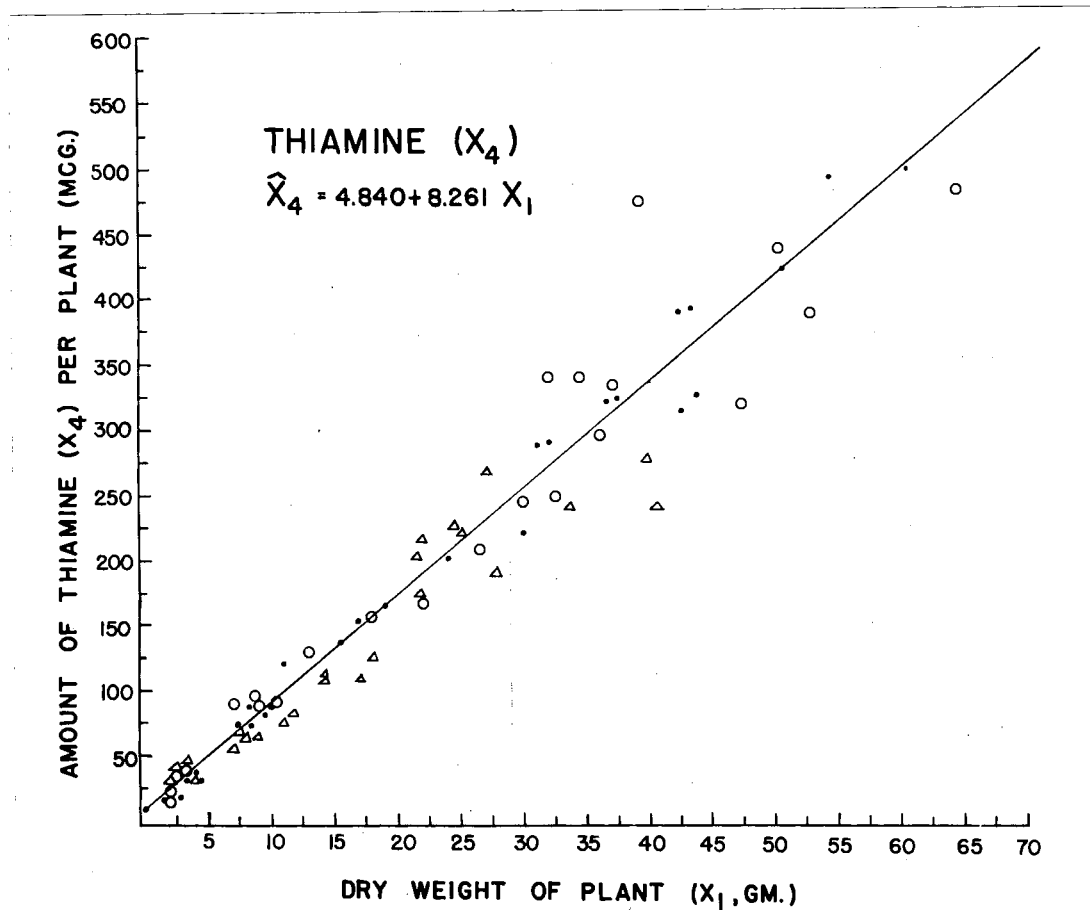
FIGURE IV



Regression of total amount of riboflavin per plant on dry plant weight in Seven Top turnip greens in three experiments.

Stillwater, fall, 1950 = Δ
 Stillwater, Spring 1951 = \bullet
 Perkins, Spring 1951 = \circ

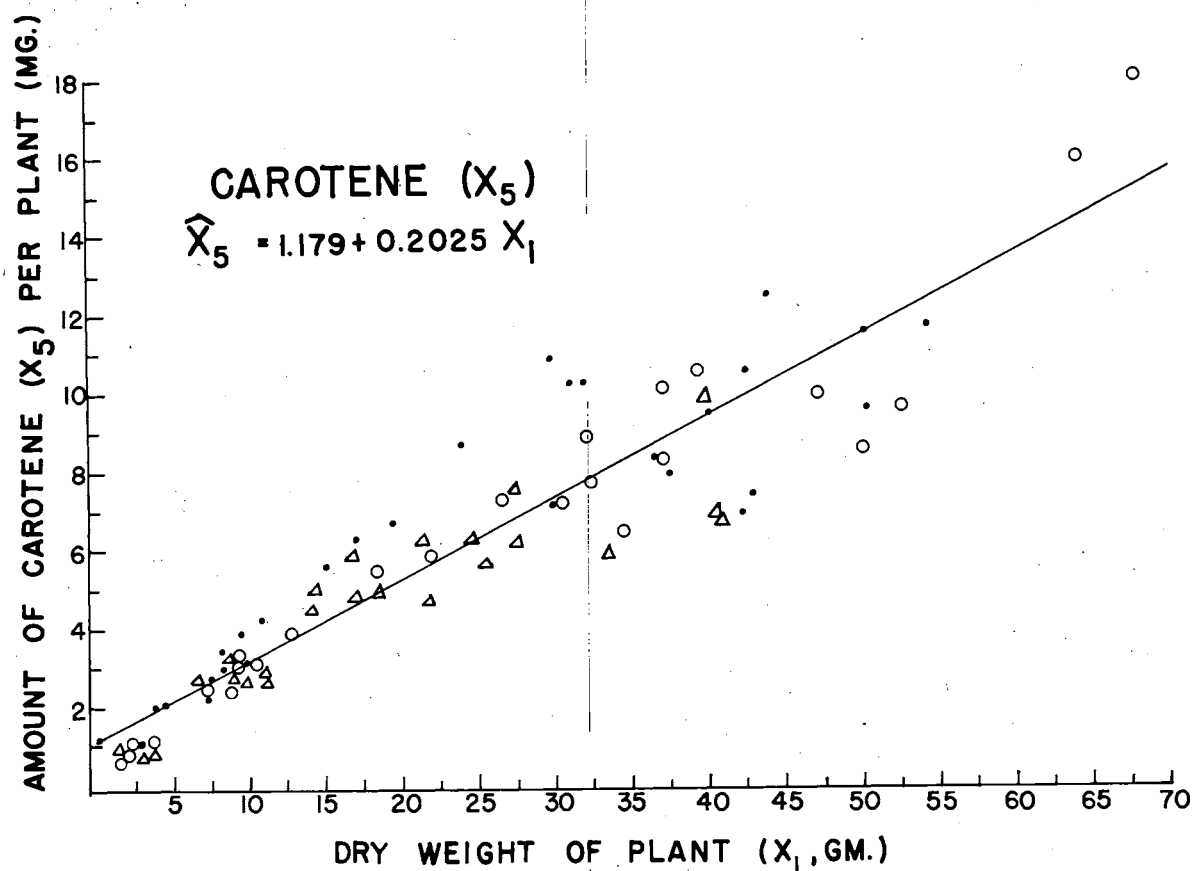
FIGURE IV



Regression of total amount of thiamine per plant on dry plant weight in Seven Top turnip greens in three experiments.

Stillwater, fall, 1950 = Δ
 Stillwater, spring 1951 = \cdot
 Perkins, spring, 1951 = \circ

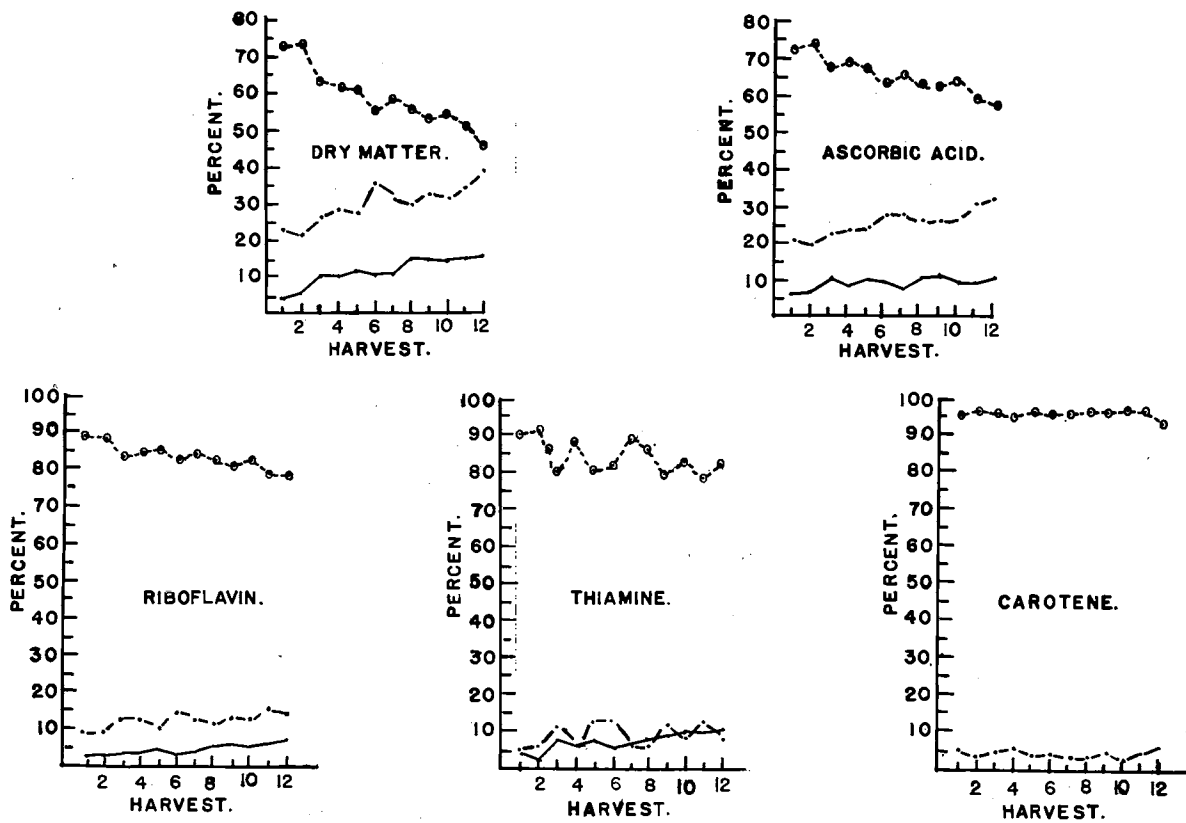
FIGURE V



Regression of total amount of carotene per plant on dry plant weight in Seven Top turnip greens in three experiments.

Stillwater, fall, 1950 = Δ
 Stillwater, spring, 1951 = \cdot
 Perkins, spring, 1951 = \circ

FIGURE VI



Mean percentage distribution of dry matter and vitamins in Seven Top turnip-green plants at 12 harvests in three experiments.

Leaf blades o---o
Midribs ·····
Roots ———

SUMMARY AND CONCLUSIONS

Relationships between the amount of dry matter in the whole plant and the whole plant content of ascorbic acid, riboflavin, thiamine, and carotene, and the distribution of these constituents among plant parts were studied in field-grown Seven Top turnip-green plants.

Three experiments were conducted at two locations during spring and fall growing seasons. Duplicate, randomly-selected samples of not less than ten plants were collected twice each week over a period of six weeks; leaf blades, midribs, and roots were analyzed for moisture, ascorbic acid, riboflavin, thiamine, and carotene.

The mean amounts of these constituents per plant in the three experiments were as follows: Dry matter, 22.97 ± 7.78 gm.; ascorbic acid, 199.4 ± 63.7 mg.; riboflavin, 386.0 ± 139.7 mcg.; thiamine, 194.6 ± 63.7 mcg.; and carotene, 5.83 ± 2.46 mg.

As the plants matured there was a highly significant increase in the total amounts of vitamin and dry matter. Correlations between plant dry weight and the total amount per plant of ascorbic acid, riboflavin, thiamine, and carotene were positive and highly significant. This was also true for plant fresh weight and total amount of each of the vitamins.

When age of plant was held constant, partial correlation coefficients for plant dry weight and the vitamin content of the

whole plant were also significant but were less than the total correlation coefficients. The decrease in correlation in the case of ascorbic acid, riboflavin, and carotene was attributed to significant decreases, with age of plant, in the amount of these vitamins per gram dry weight of the whole plant. There was no significant variation among harvests in the amount of thiamine per gram dry weight of the plant.

Regression coefficients were determined for total plant vitamin content on total plant dry matter in the three experiments; an analysis of errors of estimate showed no significant variation among experiments in the regression coefficients for ascorbic acid, thiamine, and carotene; the regression coefficient for riboflavin in one experiment differed significantly from the other two.

On the assumption of a linear relationship the following regression equations were derived from the combined data of three experiments for the estimation of the total amounts of ascorbic acid (X_2), thiamine (X_4), and carotene (X_5) in the plant from the total amount of dry matter (X_1) in the plant:

$$\text{Ascorbic acid, mg. } \hat{X}_2 = 16.667 + 7.954 X_1$$

$$\text{Thiamine, mcg. } \hat{X}_4 = 4.840 + 8.261 X_1$$

$$\text{Carotene, mg. } \hat{X}_5 = 1.179 + 0.2025 X_1$$

The following equation for the estimation of riboflavin (X_3) was derived from data in two experiments:

$$\text{Riboflavin, mcg. } \hat{X}_3 = 55.886 + 14.797 X_1$$

Correlation coefficients for the total plant content of the four vitamins with each other were positive and highly significant in all experiments.

The mean percentages of total plant dry matter in the plant parts were: in leaf blades, 54.48 ± 6.18 percent; in midribs, 30.02 ± 5.35 percent; and in roots, 11.50 ± 3.78 percent. The mean percentages of the four vitamins in the leaf blades were as follows: ascorbic acid, 65.49 ± 7.62 percent; riboflavin, 83.37 ± 4.83 percent; thiamine, 83.79 ± 7.59 percent; and carotene, 95.86 ± 1.95 percent.

As the plants matured the decrease, from 72 to 46 percent, in the percentage of dry matter of leaf blades in the combined experiments was highly significant. There were highly significant increases in dry matter in the midribs and roots; the percentage of total plant dry matter increased from 23 to 39 percent in the midribs and from 4.67 to 15.75 percent in the roots.

The percentages of ascorbic acid and riboflavin in leaf blades also decreased significantly as the plants matured, but decreases in these constituents were less than the decrease in dry matter. There were also highly significant increases in the percentages of these constituents in the roots, but increases in the midribs, were not significant.

No significant changes occurred with maturity in the percentage of thiamine in leaf blades and midribs but there was a significant increase in the percentage of thiamine in the roots.

The distribution of carotene in the leaf blades and midribs remained almost constant throughout the growing period.

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