THE EFFECT OF CERTAIN MINERAL NUTRIENTS ON THE ASCORBIC ACID CONTENT OF LEAF LETTUCE

by

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TABLE OF CONTENTS

Page

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ABSTRACT

Results from two experiments on Grand Rapids, leaf lettuce are presented. Twelve nutrient solutions were used to grow the plants in sand cultures. Determinations of ascorbic acid concentration in the fresh tissues were made colourimetrically and the results analysed statistically.

It was found that increased nitrogen gave highly significant increases in the ascorbic acid content of the plants in the winter experiment and non-significant increases in the spring experiment.

Increased phosphorus and potassium gave non-significant results. The general effect in both experiments was for phosphorus to increase and potassium to decrease the concentration of vitamin C.

Significant interactions between phosphorus and potassium in the winter experiment and between nitrogen and phosphorus in the spring experiment indicated that a balance between nutrients was as effective as the actual concentration of any element.

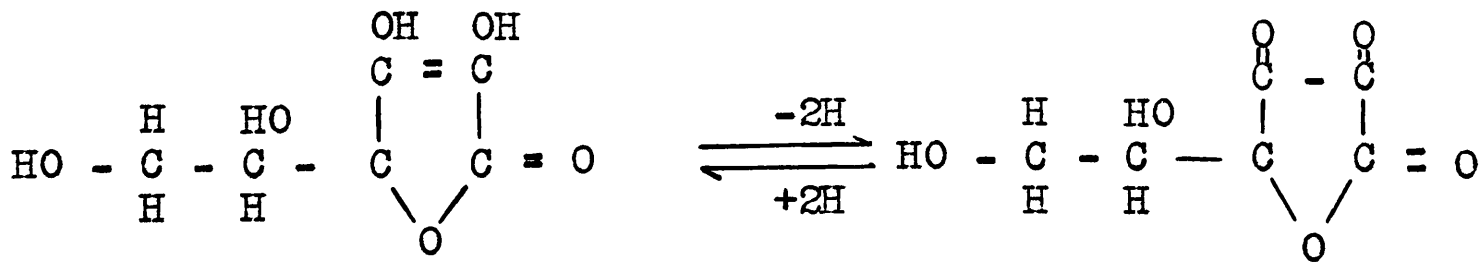
From these studies it appeared that factors other than mineral nutrition are responsible for much of the variation in the ascorbic acid content of leaf lettuce.

INTRODUCTION

Living plant tissues produce by their metabolism a substance, which when included in the diet of animals in very small quantities, protects them from the disease known as scurvy. For a long time this substance has been known as Vitamin C.

Vitamin C probably occurs in all growing and in many fully grown cells of chlorophyllous plants. Because of its antiscorbutic properties it is now called Ascorbic Acid.

The structure of vitamin C has been established: Thomas, M. (1947) states that it was the first vitamin to be synthesized by chemists. The acid is a reducing agent and can be oxidized to yield dehydroascorbic acid.



Ascorbic Acid Dehydroascorbic Acid

It has generally been reported that this vitamin exists in plant cells predominantly in the reduced form, but experiments suggest that the relative amounts of the two forms may change during development.

West, C. and Zilvia, S.S. (1944) obtained evidence that young Brambley's seedling apples stored at 3° C. synthesized Vitamin C. They suggested the possible existence in the fruit of a "chemically related precursor."

Thomas, M. (1947) suggests that the ultimate source is a sugar, and draws attention to the affinity ascorbic acid bears to hexose sugar.

It is not known whether ascorbic or dehydroascorbic acid is produced first in the plant, nor do we know if either of these particular forms is consumed in the normal metabolism of the plant.

The vitamin C content of most of our vegetables and fruits has been determined. Thomas, M. (1947) gives the range in vitamin C concentration of some of our vegetables, expressed in milligrams per 100 grams; as follows, cabbage 20 - 124, citrus fruits 20 - 100, fresh peas 5 - 40, and potatoes 11-34.

It is evident that there can be a considerable range in the concentration of the vitamin in vegetable crops and much research has been carried out to discover the factors causing variation. Somers, G.F. and Beeson, K.C. (1948) present evidence to show that light is the main factor in determining the vitamin C content of most crops. However, Platenius, H. (1945) found that a single day of cloudy, rainy weather did not decrease the Ascorbic Acid content of spinach.

Some other factors which could possibly affect the concentration of this vitamin are: temperature, rainfall, variety and mineral nutrition.

The purpose of this work is to examine the influence of the three major nutrient elements, nitrogen, phosphorus and potassium, on the ascorbic acid content of the Grand Rapids variety of leaf lettuce. The lettuce plant (Lactuca sativa L.) was chosen for this study for several reasons: 1) It is the most popular salad crop, 2) The commercial value of this crop is greater than that of any other vegetable crop, except potato, sweet potato and tomato. (It ranks second to the tomato amongst the fresh vegetables.) 3) Very little work has been done to determine the factors that influence the vitamin C content of this vegetable.

Lactuca sativa, the cultivated lettuce, is an annual. It is probably a native of Europe or Asia but is related to the wild lettuce, Lactuca scariola a North American weed.

Thompson, H. C. (1949) says that lettuce was mentioned frequently by the ancient writers, some as far back as 500 B.C. It is therefore evident that this member of the Compositae or Sunflower family has been in cultivation at least 2,500 years. Its value as a food crop is attested by its continued production.

The commercial value of the lettuce crop in the United States of America has greatly increased in the past decade. According to U.S. Bureau of Census (1949) the average yearly

value of the crop for period 1937 - 1946 was $54,804,000 as compared with the 1948 value of $103,659,000. These figures do not take into consideration the large amount of lettuce grown in home gardens for home and local consumption.

Grand Rapids, leaf lettuce was chosen for the experiment since this type is generally used in the North for winter production under glass.

The investigations were carried out by means of pot experiments and sand cultures since this is the best method of working to secure an equal distribution of the nutrient salts.

A 3 x 2 x 2 factorial experiment was designed using three levels of nitrogen, and two levels each of phosphorus and potassium; because it was desired to study the effects of various combinations of the three major elements, nitrogen, phosphorus and potassium, and also, because it was necessary to limit the size of the experiment so that the ascorbic acid determinations could be made over a relatively short period.

Two experiments were conducted, the first experiment carried out during the winter 1949-1950. This was a 3 x 2 x 2 factorial design consisting of three randomized replications of the twelve treatments. The treatments themselves were randomized within each replication. The twelve treatments were the possible combinations of three levels of nitrogen, two levels of phosphorus, and two levels of potassium. The seeds were sown on 12 November 1949 and determinations were made on 15 March 1950.

The second or spring experiment was a repetition of the first, but new randomizations were used. The seeds for this experiment was sown on 18 March 1950 and the plants were ready for analysis on 24 May 1950.

The plants for each experiment were grown in the Horticulture greenhouse in individual pots in sand cultures.

The ascorbic acid determinations were carried out colourimetrically according to methods outlined by Loeffler H.J. and Ponting, J.D. (1942), who showed that, "Ascorbic acid can be determined quickly in fruits and vegetables by disintegrating the sample with dilute metaphosphoric acid in a high speed blendor and measuring the decolouring effect of the extracted ascorbic acid on indophenol dye with a photoelectric colourimeter. The ascorbic acid is diluted with the total liquid phase present, which includes water and dissolved solids originally in the sample. Hence by using a large portion of extractant and knowing the approximate amount of liquid in the sample the ascorbic acid can be determined from as little as 3 ml. of filtrate after only one extraction. By using a large amount of 1% metaphosphoric acid as the extractant the buffering step is avoided, since the pH obtained is sufficiently low to prevent losses during blending yet sufficiently high to prevent fading of the dye reagent. The rate of fading is high with many previously recommended extraction mixtures.

"Rapid ascorbic acid determinations can be made easily on highly pigmented berries and tough dehydrated berries."

REVIEW OF LITERATURE

A. Regarding the Nutrition of the Lettuce Plant

It was deemed useful to make a review of the existing literature which concerns the production of lettuce, in order to ensure the successful growing of the plants.

Thompson, H.C. (1949) has shown that the surface soil should be well supplied with nutrients since the lettuce plant, having a small root system, is a relatively poor forager. Having reviewed the results of many field experiments he gave the following fertilizer recommendations for the crop: "On mineral soils of fairly low fertility, application of about 15 tons of stable manure, supplemented with 50 - 75 pounds of nitrogen, 100 - 150 pounds of phosphoric acid and 50 - 75 pounds of potash. On sandy soils without manure the fertilizer should contain at least 100 pounds of nitrogen, and 150 - 200 pounds each of phosphoric acid and potash."

Claypool, L.L. (1933) found that nitrogen was the limiting nutrient. However whereas nitrogen alone gave increased yields on plots where it was used alone as compared to plots receiving no treatment, still greater results were obtained when the same amount of nitrogen was used with phosphorus. Potassium when added to a treatment combining nitrogen and phosphorus did not give significant increases but when used with nitrogen alone seemed to stimulate growth.

In an article on the nutrition of horticultural plants generally, Hill, H. (1935) stated that while deficiency of an element may occur, trouble is more often caused by lack of proper balance of elements in the soil solution.

The early experimental works on plant nutrition were carried out in the field under natural conditions of soil and climate until it became evident that there was a lack of sufficient control over many factors affecting the growth of the plant.

Wallace, T. (1925) concluded that further experiments in the field would not help us to solve the nutrition problems of fruit plantations and initiated the use of sand culture methods.

Methods of growing plants in solutions and in sand cultures were set forth by Shive, J.W. and Robbins, W.R. (1938). They recommended the constant drip method of supplying the nutrients. A more complete discussion of the use of sand cultures was given by Robbins, W.R. (1946) who now favoured the daily application of nutrients.

MacLeod, P.R. (1943) and Chan, A.P. (1946) used a system of weekly feeding with more concentrated nutrient solutions, pure water being supplied between feedings if required by the plants. These workers recommended that the sand in the crocks be given a thorough flushing to leach out any accumulated salts, etc., at least fortnightly.

Very few workers are on record as having done work on the nutrition of lettuce in sand cultures. Woodman, R.M. (1940) who worked on May King in England found that nitrogen and phosphorus gave good results but that potassium gave practically no response, except when reduced to very low concentrations, in which case it delayed maturity. Chan, A.P. (1946) obtained results that agreed with those of Woodman with the exception that he found that there was a critical level of nitrogen beyond which there was deleterious effects.

B. Regarding the Effect of Mineral Nutrition on the Ascorbic Acid Content of Vegetables and Fruits

The effect of fertilizer treatments on the concentration of ascorbic acid (vitamin C) of fruits and vegetables has been the subject of considerable research. Much of the evidence obtained has been contradictory. Since light is the factor that has the greatest effect on vitamin C content and since shading affects the ascorbic acid concentration in fruits, it appears likely that nutrition would affect the concentration of this vitamin differently in fruits than in foliage.

This theory was probably best exemplified by the work of Wittwer, S.H. and Hibbard, Audrey D. (1947) who found that large quantities of nitrogen applied as a fertilizer caused a depression in the ascorbic acid content of peaches. Somers, G.F., Hamner, K.C. and Kelly, W.C. (1949) found that with tomatoes grown in sand cultures shaded fruits contained less ascorbic acid than comparable fruits exposed to sunshine.

Since the effects of nutrition on the ascorbic acid concentration of fruits is confounded with light in that increased growth of plants caused by higher nitrogen supply causes a shading of the fruits and thus lowers the ascorbic acid content of the fruits, it has been deemed necessary for the purpose of this work to cite here only the works of those experimenters who have used vegetables of a leafy nature as lettuce.

Tressler, D.K., Mack, G.L., and King, C.G., (1936) found that the soil on which spinach is grown apparently has a somewhat important influence on the ascorbic acid content of the leaves, while there is a varietal difference also. They found that spinach grown on upland soils (mineral) averaged 50% higher in ascorbic acid than that from muck soil. Ijdo, Jan B.H. (1938) reported, from his work on spinach at the University of Utrecht, Holland, that the ascorbic acid content depended on the amounts of nitrogen x potassium in the soil. Large amounts of nitrogen in the soil resulted in greater ascorbic acid concentration. He also found that increased potassium gave an increase in ascorbic acid. Other salts used in his works, such as, phosphorus, calcium and magnesium had very small influence. From the results of this work he concluded that N x K stands in close interrelation physiologically. Potassium deficiency has the effect of nitrogen excess and potassium excess acts like nitrogen deficiency. He also concluded that vitamin C is a product of assimilation or at least is closely connected with this process.

Vitamin C content was found to increase but little at low levels of nitrogen as a result of the addition of potassium fertilizers, whereas at high nitrogen levels a rapid increase was observed.

Isgur, B. and Fellers, C.R. (1937) in their work with swiss chard and spinach found that increases in yield of swiss chard caused by fertilizer application were accompanied by increases in vitamin C content. They found that high nitrogen treatments resulted in the highest yields of ascorbic acid. Their work on swiss chard agreed with the work of Ijdo (1936) who worked on spinach. However, contrary to the findings of Ijdo, they found that spinach did not give similar responses.

Sugarawa, Tomota (1938) concluded from the results of his experiments, also on spinach, that the amount of fertilizers applied to the soil greatly influenced the amount of ascorbic acid in the plants. Ascorbic acid concentration was found to be high when applications of nitrogen, phosphorus and potassium were high. He found that vitamin C content of plants was highest when the plants had attained their full growth.

Burrell, B.C. et al (1940), working with cabbage at Ohio State University, found from their experiments that high nitrogen or a complete fertilizer gave rise to a higher ascorbic acid content in cabbage than did six other treatments investigated.

The results from the 2 x 2 x 2 x 2 factorial experiment on turnip greens, carried out at four locations by Reder, Ruth (1943) are listed below.

Nitrogen - gave increases in ascorbic acid at two locations (significance at one location) and gave significant decreases at two locations.

Phosphorus - gave a significant increase at one location and non-significant decreases at three locations.

Potassium - gave a decrease in ascorbic acid at all locations with significance at three locations.

Calcium - no significant effects.

N X P - highly significant at one location.

N X Ca - gave decreases at all locations.

Perhaps the results of this experiment best exemplify the results obtained by workers on the subject.

Bernstein, L., Hamner, K.C. and Parks, R.Q. (1945) also working with turnip greens reported that only minor differences in ascorbic acid concentration could be associated with the supply of macronutrient elements when plants were grown in sand culture, except when these elements were completely absent.

The role of manganese was studied by Harmer, P.M. and Sherman, G.D. (1944) who discovered that soil deficient in manganese gave a lower concentration of ascorbic acid in foliage of plants than soil that had been corrected for this element. They found that application of manganese to

deficient soil gave a significant increase in ascorbic acid concentration. This may be explained by the fact that manganese played a role in maintaining a proper oxidative level for ascorbic acid in the plant tissue. A lack of sufficient manganese generally caused the oxidized form of ascorbic acid to increase and the reduced form to decrease. It was not clear what level of significance was adopted by Harmer and Sherman as in a foot note they stated that at least in one set of data of their analysis of variance they showed signiciant differences at a 17% level.

Wynd, F. L. and Noggle, G.R. (1945) and (1946) presented evidence in 1945 to show that the ascorbic acid concentration in the oat plant is connected to the nitrogen in the soil. However in 1946 they carried out a similar experiment using rye instead of oats and found opposite results. Somers and Beeson (1948) reviewed the work of Noggle and Wynd and pointed out several faults in technique.

Somers, G.F. and Beeson K.C. (1948) summarized the conclusions reached by Hamner, K.C. (1945) as follows: "It seems probable that variations in 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 practises will be found to have little practical significance."

In regards to the foliage of leafy vegetables it is evident that mineral nutrition, at least in some instances, have given significant variations in the ascorbic acid (vitamin C) concentration.

It is unfortunate that the literature has not reported any instance of experiments having been carried out to determine the effect of mineral nutrition on the ascorbic acid concentration of lettuce.

C. Design of Greenhouse Experiments

Cox, G.M. and Cochran, W.G. (1946) presented a review giving the design and methods of analysis of the more recent statistical designs for use in experiments to be conducted in the greenhouse.

Among the many designs reviewed was the "Factorial Design." This design makes it possible to separate out the source of variation due to the interaction of the treatments as well as the variation due to the treatments individually. This design is very useful in nutrition experiments and was chosen for this work.

OBJECTIVES

These investigations were undertaken to determine what effect the three major nutrient elements, nitrogen, phosphorus and potassium, had upon the ascorbic acid (Vitamin C) content of lettuce. They continued a series of investigations which have been conducted at Macdonald College over a period of years, relating to the mineral nutrition of vegetable crops.

EXPERIMENTAL TECHNIQUE

Grand Rapids, a variety of leaf lettuce, was used as the crop under test. This variety is a non-heading type of lettuce with a small heart and has most of its leaves exposed to sunlight. This habit of growth accounts for the presence of chlorophyll in all the leaves of the plant and the absence of the blanched wrapper leaves present in the head lettuce varieties.

Grand Rapids leaf lettuce can be grown successfully under conditions prevailing in the Horticultural greenhouse at Macdonald College. This greenhouse is a large, undivided structure and is used in part for the commercial production of a number of cool-season crops. The greenhouse was kept free of insect infestation during the periods of these experiments by regular fumigation with Nicofume.

Under the conditions prevailing in the greenhouse the plants under test made steady growth and remained free from disease and insect damage.

The growth of the plants as compared to that of other crops in the house at the same periods was good. The conditions in the greenhouse were favourable for plant growth.

Climatic conditions at the time of the winter experiment were not optimum owing to the very short periods of sunshine during January and February (see Figure IIIa).[1]

WINTER EXPERIMENT

Introduction

This experiment was undertaken to test the effects of different combinations of the levels of the three macronutrients on the growth of the lettuce plants. It was designed to gather information concerning the growing of lettuce under glass and to find out if the proposed levels of the three major elements would produce a satisfactory crop of lettuce for the tests.

It was hoped also to ascertain if waxed clay pots would be satisfactory containers to grow the plants in. The use of these small pots would reduce the space required in the greenhouse, would require less materials and cut down the amount of labour involved.

1) "a" indicates Tables and Figures placed in appendix.

Design of Experiment

This experiment was laid out in a 3 x 2 x 2 factorial design with three replications. This design was chosen because it permits the study of the interactions between the treatments as well as the main treatment effects.

In setting up a statistical design of this nature it was necessary to ensure that eachlevel of an element occurred in combination with each level of the other two elements in each replication of the experiment. Therefore in a 3 x 2 x 2 factorial each of the three levels of nitrogen must occur with each of the two levels of phosphorus and with each of the two levels of potassium. The arrangement was made in a manner to ensure that the two levels of phosphorus were each combined with each level of potassium. It will be seen that such a design required twelve combinations of the elements. These combinations are referred to hereafter as nutrient solutions, such as nutrient solution 3 - 2 - 1, etc.

In the expression 3 - 2 - 1 the first figure "3" represented the third or highest level of nitrogen, the second figure "2" represented the second or higher level of phosphorus while the last figure "1" represented the first or lower level of potassium.

Another desirable feature of the unconfounded factorial design lies in the fact that in the analysis each main effect and interaction is measured by using all the data. This is possible through repeated grouping of the data.

Because of temperature and ventilation gradients in the greenhouse randomization was necessary, as in a field experiment, in order to obtain a valid estimate of error. In this experiment the replications were randomized and the treatments were randomized within each replication.

The pots were placed on a raised centre bench in the greenhouse according to the layout recorded in Table I.

Temperature was controlled manually and therefore a constant temperature was impossible to maintain. The greenhouse was heated by means of a system of steam pipes under the benches and another system of steam pipes in the air space about three feet above the beds. This arrangement ensured that the temperature of the air and soil remained nearly the same.

Ventilation was provided when required through ventilators which extended the entire length of the greenhouse. These ventilators were so arranged that a section could be opened by means of a worm gear to any desired distance. This peak ventilation helped to guard against draughts and cross currents of air. Owing to the relatively short periods of sunlight during the winter months ventilation had to be held to a minimum.

TABLE I

Randomization of pots in winter experiment[1]

Replication 1		
1 - 2 - 2	3 - 1 - 2	2 - 1 - 1
2 - 1 - 2	3 - 2 - 1	3 - 1 - 1
3 - 2 - 2	1 - 2 - 1	1 - 1 - 2
1 - 1 - 1	2 - 2 - 2	2 - 2 - 1
Replication 3		
2 - 2 - 2	1 - 2 - 1	3 - 1 - 2
3 - 1 - 1	2 - 2 - 1	2 - 1 - 1
3 - 2 - 1	2 - 1 - 2	3 - 2 - 2
1 - 1 - 2	1 - 2 - 2	1 - 1 - 1
Replication 2		
2 - 1 - 2	2 - 1 - 1	3 - 2 - 2
3 - 1 - 2	1 - 2 - 2	3 - 1 - 1
2 - 2 - 2	1 - 1 - 1	2 - 2 - 1
1 - 2 - 1	3 - 2 - 1	1 - 1 - 2

[1]Figures in formulae represent the levels of N - P - K.

METHODS AND MATERIALS

Seedling Production

The seeds were sown in washed sand in flats on 12 November 1949.

The flats were kept moist to ensure good germination and the young plants began to emerge on 20 November 1949. The seedlings were grown in the moist sand until they had developed true roots and were then given their first application of a starter solution on 27 November 1949.

The starter solution, see Table II for composition, was applied directly to the soil as were all nutrient solutions in these experiments. This method was employed to guard against injury to the leaves by the chemicals. The starter solution was applied as required until 8 December when the seedlings were transplanted to the individual pots.

After being transferred to the waxed pots the plants were given three feedings of the starter solution at four day intervals to allow them to become established before the nutrient solutions were begun on 20 December.

At the time of transplanting an attempt was made to select seedlings of nearly equal size and vitality.

TABLE II

General nutrient solution for small seedlings[1]

Chemical	Formula	Amount
Calcium nitrate	$(CaNO_3)$	12.79 gms.
Magnesium sulphate	$MgSO_4 \cdot 7H_2O$	5.09 "
Superphosphate (20%)		6.34 "
Muriate of potash (48%)		2.00 "
Boric acid	H_3BO_3	0.032 "
Magnesium sulphate	$MnSO_4 \cdot 4H_2O$	0.0046 gms.
Water	H_2O	5.00 gallons

[1]Hill, H. (1940)

The individual containers for growing the plants were seven inch, three-quarter pots of unglazed clay which had been made impervious to moisture by two coats of paraffin wax. This was done by dipping the clean dry pots in hot melted wax. The first coat was allowed to harden before the second application was made.

Drainage was provided for by a single hole centrally located in the bottom of the pot. A wad of pyrex glass wool was inserted in the drainage hole of each pot to prevent the sand escaping while providing for free drainage.

The capacity of the pots used in the experiment was approximately 2350 ml.

The Growing Medium

The pots were filled to within three-quarters of an inch from the top with a mixture of pure quartz sand and finer river-washed sand. The analysis of the sand used is given in Table III.

The sand mixture was thoroughly washed with hot running water and allowed to stand for twenty-four hours in hot water after which it was given a second flushing with hot water. Finally the sand was washed with distilled water before being put into the pots. Washing was accomplished by placing 100 pounds of the sand mixture in a barrel and forcing the hot water through it from a hose. The overflow from the barrel carried away the impurities. Each washing required about six hours during which time the sand was stired and the water emptied from the barrel completely several times.

The sand was put into the pots with another pot so as to avoid contact with metal and after the pots were filled, the sand was flushed twice with water before the plants were transplanted.

The nutrient solutions were first used on 20 December 1949 and thereafter application of nutrients was made every sixth day throughout the growing period.

The sand in the pots was flushed thoroughly every two weeks to remove any accumulated nutrient ions and to free the growing medium of algal growth which began to appear in February. This algae was undesirable because it was feared that its decomposition might add organic matter to the sand.

TABLE III

A physical analysis of the sand used in winter experiment

(U.S. Standard Sieve Series)

Particle size	Percentage by weight
Not passing a 20 mesh sieve	50.26%
Passing a 20 mesh but not a 40 mesh sieve	40.17%
Passing a 40 " " " " 60 " "	7.94%
Passing a 60 " " " " 80 " "	0.85%
Passing an80 " sieve	0.78%

The Composition of the Nutrient Solutions

The composition of the basic solution was a modified form of the one recommended by Hill, H. (1940) and used by several workers at Macdonald College in the series of nutritional experiments on vegetable crops; including, Chan, A.P. (1946) and in a revised form by Campbell, J.D. (1950). The concentrations of the minor elements were revised and

micro-elements were added in accordance with recommendations of Arnon, D.I. (1938) Table IV.

TABLE IV

Parts per million of elements in basic nutrient solution

Element	Compound used to supply element	p.p.m.
Nitrogen[1]	KNO_3 & NH_4NO_3	626
Phosphorus[2]	KH_2PO_4	122
Potassium[3]	KH_2PO_4& KNO_3	464
Magnesium[4]	$MgSO_4.7H_2O$	96
Calcium	$CaCl_2.2H_2O$	400
Manganese	$MnSO_4.4H_2O$	0.5
Boron	H_3BO_3	0.5
Iron	$Fe(C_6H_5O_7).3H_2O$	5.0
Zinc	$ZnSO_4.7H_2O$	0.05
Copper	$CuSO_4.5H_2O$	0.02
Molybdenum	$Na_2MoO_4.2H_2O$	0.01
Chromium	$K_2Cr_2O_7$	0.01
Cobalt	$CoCl_2.6H_2O$	0.01
Tungsten	$Na_2WO_4.2H_2O$	0.01

[1]The three levels of nitrogen were 626 p.p.m., 416.3 p.p.m. and 208.7 p.p.m.

[2]The two levels of phosphorus used were 122 p.p.m. and 40.7 p.p.m.

[3]The two levels of potassium used were 464 p.p.m. and 154.7 p.p.m.

[4]The concentration of all other elements were held constant for all nutrient solutions. The twelve nutrient solutions were made up by combining the levels of nitrogen, phosphorus and potassium cited above.

The concentration of elements in the twelve nutrient solutions and methods of calculating quantities are recorded in the appendix (Table IIa).

Methods of Feeding Plants

Stock solutions of each element were made up and placed in glass bottles with burettes attached to simplify measuring of the required quantities. The four micro-elements chromium, cobalt, molybdenum and tungsten were placed in one bottle. For concentrations of stock solutions, refer Table Ia.

Twelve feeding bottles were numbered, one for each nutrient solution, and graduated so that the required volume of nutrient solution could be made up. To prepare a nutrient solution approximately 150 ml. of water were placed in the feeding bottle and the required amount of each stock solution was added then the volume was made up to 300 ml. which was the amount required to feed the three plants receiving that particular solution. The twelve nutrient solutions were made up and carried to the greenhouse bench. Each feeding bottle was taken in turn and 100 ml. of solution was applied to the sand of the plant in each replication receiving the treatment. It was found that 100 ml. of solution would completely saturate the sand in the pot and permit some slight drainage.

A plastic sprinkler was used to apply the solution so as to give an even distribution over the sand. This sprinkler was rinsed between solutions.

For the duration of this experiment the sand in the pots was thoroughly flushed immediately before every second feeding and water was supplied between feedings if it was required.

The nutrient solutions were supplied every sixth day.

Control of Greenhouse Temperature

The complete control of temperature in the greenhouse was found to be impossible as the structure is large, undivided and used in part for commercial purposes. The temperatures during January and February varied as follows:

Day temperatures	55 - 80° F.
Night temperatures	55 - 65° F.

Plant Growth

Growth during January and February was slow because of lack of sunlight. This was true of all crops grown in the greenhouse. However, progress was steady and the plants were ready for analysis on 15 March 1950. At the time of the analysis all the plants were healthy and colour of the leaves was good. There was no evidence of diseases such as tipburn in the crop.

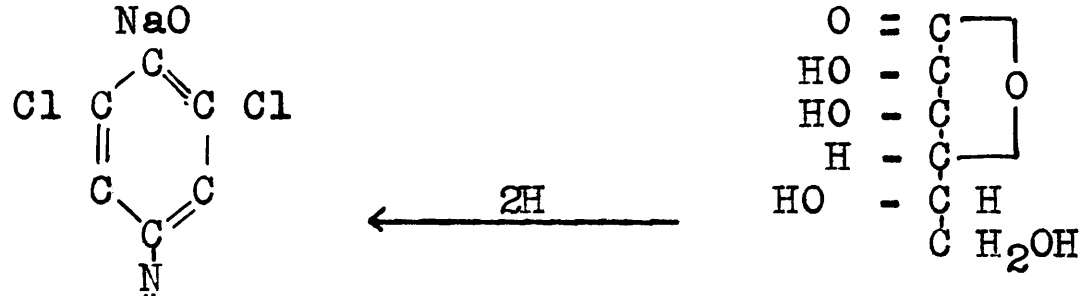
The plants grown on the nutrient solution combining the lowest levels of all major elements were slightly paler in colour than other plants but no noticeable differences were evident in plants receiving other treatments.

Ascorbic Acid Determinations

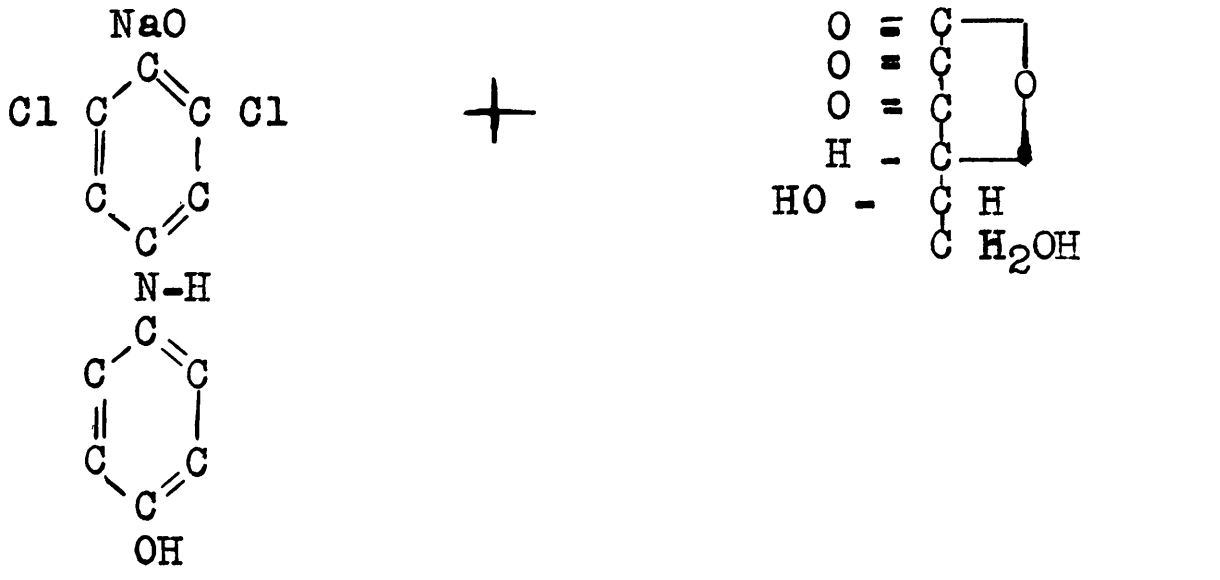
Ascorbic acid, or vitamin C, was extracted from the plant tissue with metaphosphoric acid. The sodium salt of 2-6 dichlorophenolindolphenol, which is blue in its oxidized state and colourless when reduced, was used for the chemical estimation of the ascorbic acid. The reaction is probably as shown.

Probable reaction in reduction of 2-6 dichlorophenolindolphenol

Blue dye ⟵ 2H ⟵ **Ascorbic acid**



Colourless form + **Dehydroascorbic acid**



The colourimeter used to determine the percentage light transmission through the dye and reduced solution was a Hellige-Diller, Bio-Photocol, photoelectric model No. 500.

The machine was adjusted so that a wavelength of 520 mμ was used for the determinations.

The Calibration Curve

The first step in the procedure of determining the ascorbic acid concentration was to prepare a calibration curve using known concentrations of ascorbic acid solution to reduce a known concentration of the oxidized form of the dye.

The solutions used in calibrating the colourimeter were:

1. A 1% metaphosphoric acid solution.

2. A solution of the sodium salt of 2-6 dichlorophenol indol phenol made up as follows:

20 mg. of $NaOC_6H_6N_4$: $C_6H_2(Cl_2)$: O in 100 ml. of water.

The dye was dissolved in boiling water, filtered, and the solution made up to volume with water.

3. Ascorbic acid solution made by dissolving 100 mg. of ascorbic acid in 100 ml. of 1% metaphosphoric acid. One ml. of the solution was diluted to 100 ml. with 1% metaphosphoric acid and this dilution was used in calibrating the colourimeter.

The calibration curve was prepared by measuring the change in the transmittance of radiant energy due to the reaction of the ascorbic acid on the dye. For these readings

a total of 10 ml. was used in the cuvettes each time they were placed in the colourimeter.

The first step was to determine the transmittance due to completely decolourizing the dye solution (5 ml.) used for each reading. To do this 5 ml. of the dye solution was placed in the cuvette and diluted to the required 10 ml. with 1% metaphosphoric acid, the mixture was shaken and the cuvette was placed in the machine and a reading was taken. Next the dye in the cuvette was completely reduced by adding a few grains of ascorbic acid and a second reading was taken. The difference between these two readings gave the measurement of the transmittance allowed by the reaction of the ascorbic acid on the dye.

This procedure was followed using 5 ml. of dye solution and different volumes of the diluted ascorbic acid solution, the variation in transmittance was measured and a straight line graph constructed (see Figure Ia).

Preparation of the Sample

The determinations were made on 15 March 1950. This was a bright sunny day and all determinations were completed during the sunny hours. The plants were allowed to be in sunlight for two hours before the analysis was begun.

Each plant was removed from the greenhouse individually and determination made immediately to ensure that the effect

of reduced sunlight in the laboratory would not reduce the ascorbic acid concentration.

The plant was cut at the base of the leaves and a 25-gram sample was taken. In order to ensure that this sample was representative of the whole plant and that it should include both outer and central leaves the plant was laid on a table and first cut in half. It was then cut pie-fashion to make up the required weight.

Duplicate samples were taken in a number of cases to check the results being obtained, the remaining portion of the plants was used for these.

Extracting the Ascorbic Acid

The 25-gm. sample of plant tissue was placed in a clean Waring blendor with 200 ml. of 1% metaphosphoric acid and blended for three minutes. The resultant mixture was suction filtered, a clean test tube was placed in the filter flask so that it could be removed with the filtrate. It was found that by discarding the first test tubeful of filtrate and using the second a clearer solution was obtained.

A 10 ml. volume of this filtrate was placed in a 50 ml. volumetric flask and made up to volume with 1% metaphosphoric acid. From this diluted solution of the filtrate 5 ml. was taken for the reading.

Adjusting the Colourimeter

The colourimeter was adjusted to read 80% transmittance when a water blank was placed in the path of the light. This point was taken as the machine could not be adjusted to read 100% owing to fluctuations in the electric current being used. The adjustment of the colourimeter was checked with the water blank before each reading was taken during the determinations.

The Determinations

To make the determination 5 ml. of the dye solution was placed in the cuvette and the 5 ml. volume of the sample solution was added. The cuvette was rotated to mix the solutions and the reading was taken within 15 seconds of the time when the two solutions were combined. Actually no change in readings was noticed even after the solutions had been mixed for one hour, it was deemed advisable however to make the readings as quickly as possible.

After the reading was taken the dye was completely decolourized with a few crystals of ascorbic acid and a new reading was taken. The difference between this reading and the setting of 80% transmission was due to colouring matter other than dye in the solution and this difference was added to the original reading for the sample, to correct for the colouring matter in the sample. Usually this difference was very slight.

The readings are recorded in appendix as Table IVa.

Conversion

The concentration of ascorbic acid in plant tissue is usually expressed in mg. per 100 gm. of tissue (fresh weight). The conversion of the colourimeter readings to mg of ascorbic acid per 100 gm. of plant tissue was accomplished by using the calibration curve prepared previously, Figure Ia appendix, to convert the readings to μ gm. per ml. of solution and then by calculation to determine the concentration of ascorbic acid in mg. per 100 gm. of plant tissue.

The calculations are given in detail in the appendix.

Record and Analysis

The results of the determination are recorded in the appendix. From the Analysis of Variance, Table VIa and Fig. I, it was found that the only treatment that gave significant variation in the ascorbic acid concentration was nitrogen. This variation due to nitrogen reached the P. 01 level of significance and the effect was found to be linear.

TABLE V

Comparison of ascorbic acid concentrations
(Totals for experiment)

Level	N^1	P^2	K^2
Low	317.34	503-82	524.07
Medium	350.87		
High	357.66	522.05	501.80

Necessary Difference for comparisons between levels of nitrogen: P. 01 = 29.58, P. .05 = 21.7

1) Figures represent sum of 12 plants.
2) Figures represent sum of 18 plants.

It is evident from the table above that there was a highly significant difference in ascorbic acid concentration between the lowest and each of the other two levels of nitrogen. Although the "F" test showed that the effect of nitrogen was linear the increase between the second and third levels when compared by the "t" test did not show significance.

From the analysis it was shown that there was a highly significant interaction between the phosphorus and potassium treatments.

TABLE VI

Summary of results phosphorus x potassium
(Totals)

		1K	2K
1N + 2N + 3N	1P	270.81	233.01
	2P	253.26	268.79

It would appear from Table VI that the highest concentrations of ascorbic acid were obtained when either the two low levels or the two higher levels of these elements were combined, and that decreasing or increasing the one while the other was held constant had the effect of decreasing the concentration of ascorbic acid in the plant tissues. From the information obtained from this experiment it would appear that it was the balance between the two that was the

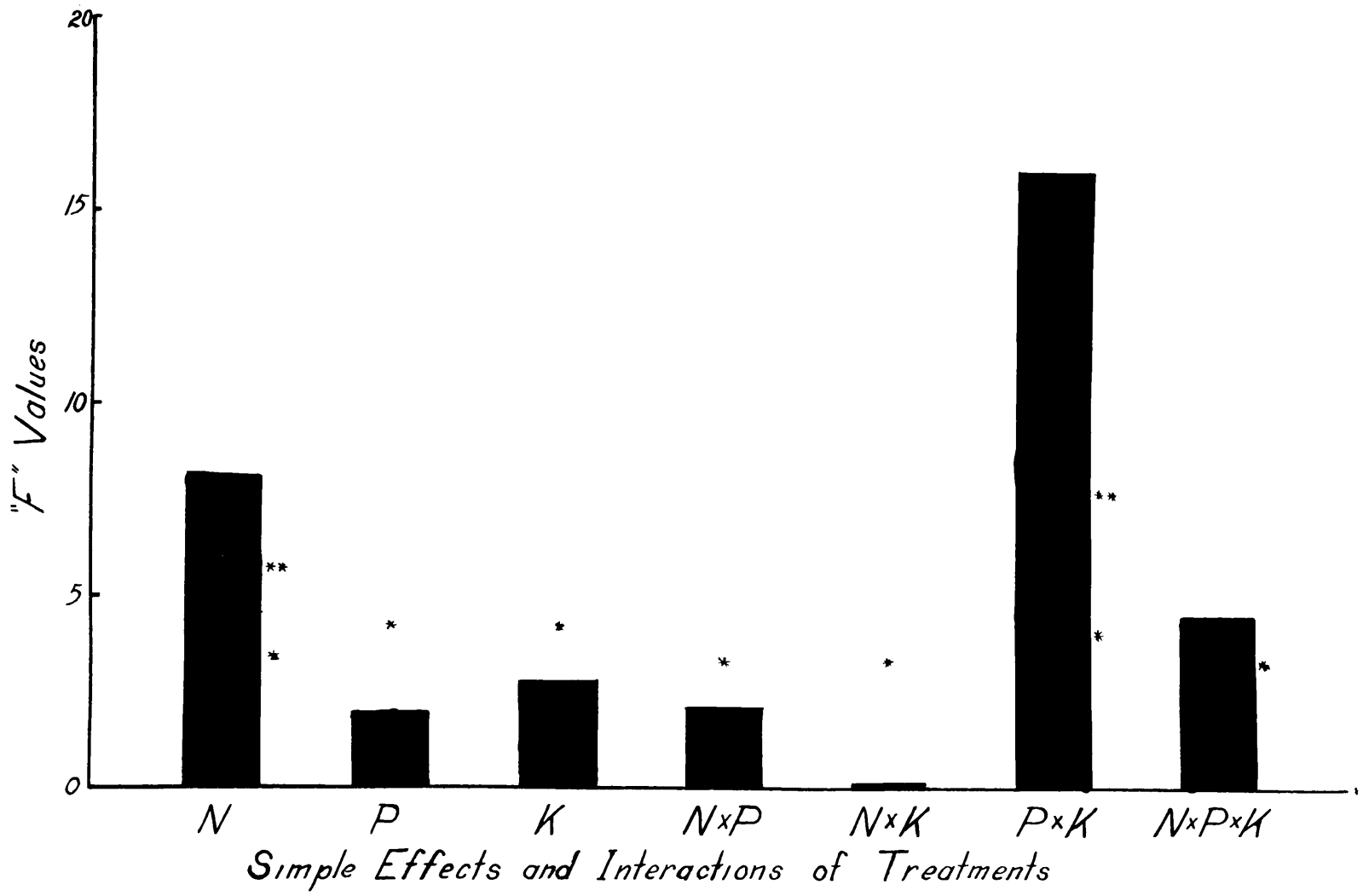


FIGURE I

Winter Experiment

**P .01 level of significance

*P .05 level of significance

determining factor rather than the actual amounts of each supplied, since the variation due to neither element was itself significant.

TABLE VII

Summary of results of N X P X K
(Totals)

	1N		2N		3N	
	1K	2K	1K	2K	1K	2K
1P	88.56	63.90	86.49	82.71	95.76	86.40
2P	76.41	88.47	89.37	92.30	87.48	88.02

N.D. for treatment totals at P .01 Level = 14.77

N.D. for treatment totals at P .05 Level = 10.84

From Table VII it appeared that the significant second order interaction was due mainly to the interaction of phosphorus and potassium and that it was the balance between the levels of these two elements that was necessary for the production of the maximum concentration of ascorbic acid.

The interaction of P X K was more noticeable when the highest and lowest levels of nitrogen were used. When the medium level of nitrogen was considered, the highest production of ascorbic acid was obtained when the nitrogen was in combination with the higher levels of the other two elements.

Conclusions Drawn from the Winter Experiment

The aim of this work has been to determine whether the ascorbic acid concentration in lettuce leaves can be increased by varying the concentrations of the major nutrient elements supplied to the plants.

The plants used for the experiment was Grand Rapids, leaf lettuce. The tests were carried out during the winter of 1950 and while plant growth was slow owing to the limited amount of sunlight during the period, the plants made steady growth and remained healthy and free from insect injury.

A review of the statistical analysis of the results of the ascorbic acid determinations revealed that the variation due to treatments was highly significant and that highly significant increases in ascorbic acid were obtained by increasing the levels of nitrogen.

Neither phosphorus nor potassium gave significant results. However the general effect of phosphorus was to increase the ascorbic acid while that of potassium was to decrease the concentration of this vitamin. The interaction between these two elements was highly significant and a review of the results appeared to indicate that a balance between these two elements was more effective in increasing the ascorbic acid concentration in the plant than was the actual concentration of either element in itself.

Since the interaction of the three elements was significant, a study of the results was made which revealed that

this was mainly due to the interaction of phosphorus and potassium. It was noted, however, that when the medium level of nitrogen was employed the maximum results were obtained when it was in combination with the highest level of phosphorus.

From the results of this particular experiment it would appear that nitrogen fertilizer is effective in increasing the ascorbic acid concentration of the lettuce plant. It was also shown that a balance between the major elements was required. This was more particularly true in the case of phosphorus and potassium.

SPRING EXPERIMENT

Introduction

This experiment was conducted to verify the results obtained in the winter experiment. The decision to carry out this work was taken in view of the many questions that had arisen from the results of the earlier tests.

Since a considerable increase in the amount of sunlight could be expected for the period during which the plants for this experiment would be grown, the questions arose whether the increased photosynthesis would require the utilization of more nitrogen and whether this increase in photosynthesis would give a greater production of ascorbic acid. In other words would the utilization of more nitrogen give rise to greater increase in ascorbic acid concentration.

The spring experiment followed the same general pattern of the first experiment with minor changes in technique. However, the environmental conditions of sunlight and temperature were decidedly different from those of the winter experiment.

A graph showing the hours of sunlight for the entire duration of these experiments is given in the appendix (Figure IIIa).

Design of Experiment

A 3 x 2 x 2 factorial design was again chosen for this experiment for reasons stated previously. For this experiment

however two plants were grown in each replication for each treatment, and the results recorded for each treatment is the average of the determinations from the two plants. This made for greater accuracy as the results were based on the data from twice the number of plants used in the previous tests.

The three replications of the twelve treatments were laid out on the same greenhouse bench. The randomization is shown in Table VIII. Each treatment was applied to two plants in each replication, thus making a total of six plants per treatment. The two plants receiving the same treatment were placed side by side in each replication.

TABLE VIII

Randomization of crocks in spring experiment[1]

Replication 2		
3 - 2 - 2	3 - 1 - 1	2 - 2 - 1
2 - 1 - 2	2 - 2 - 2	1 - 1 - 1
1 - 2 - 1	3 - 2 - 1	3 - 1 - 2
2 - 1 - 1	1 - 2 - 2	1 - 1 - 2
Replication 1		
1 - 2 - 2	2 - 1 - 2	2 - 2 - 2
3 - 2 - 1	1 - 2 - 1	1 - 1 - 1
1 - 1 - 2	3 - 1 - 1	3 - 1 - 2
2 - 2 - 1	3 - 2 - 2	2 - 1 - 1
Replication 3		
2 - 1 - 2	1 - 2 - 2	3 - 2 - 2
3 - 1 - 1	2 - 1 - 1	2 - 2 - 1
1 - 2 - 1	3 - 2 - 1	1 - 1 - 1
2 - 2 - 2	1 - 1 - 2	3 - 1 - 2

[1]Two plants per treatment per replication.

Methods and Materials

The lettuce plants used for the determinations in the experiment were grown from seed from the same sample used in the first experiment.

The pots used in this experiment were glazed earthenware crocks with vertical sides. The crocks were approximately 17½ cm. in diameter by 18½ cm. deep and held about four litres. Drainage was taken care of by two drainage holes, one in the bottom of the crock and the other on the side at the base. To prevent sand from escaping and to ensure proper drainage these holes were stoppered with pyrex glass wool.

The sand used was river-washed sand. This sand was thoroughly washed free of impurities with hot water, as described in the winter experiment and similar precautions taken to prevent contamination as before. The sand used was somewhat finer than that used in the first instance and an analysis is given in Table IX.

TABLE IX

A physical analysis of the sand
(U.S. Standard Sieve Series)

Not passing a 20 mesh sieve	19.1%	by weight
Passing a 20 mesh but not a 40 mesh sieve	72.9%	" "
Passing a 40 mesh but noa a 60 mesh sieve	7.0%	" "
Passing a 60 mesh but not a 80 mesh sieve	0.9%	" "
Not passing an 80 mesh sieve	0.1%	" "

Reasons for the Changes in Methods and Materials

In the earlier experiment it was noted that the wax on the pots had a tendency to accumulate algal growth, furthermore the slight taper to the sides of these pots made it difficult to flush the algae from the sand as it tended to give the material support. Some trouble was also encountered with the peeling of the outer layer of wax from the outside of the pot during the later weeks of the long period required to grow the lettuce plants.

It was also felt that the single drainage hole was insufficient and drainage was slowed up, particularly during the flushing operations. Conditions in the greenhouse at the time of planting were more conducive to growth than previously and it was felt that nutrient solutions should be supplied every five days instead of every sixth day and that a better control of nutrient could be maintained if the growing medium was flushed before each feeding rather than before every second feeding. It was decided therefore that the pots with the single hole would not permit sufficiently free drainage.

Sand of similar composition to that used in the winter experiment was not available at the time this experiment was begun but it was felt that the finer river washed sand used, would be more retentive of moisture during the period of growth when water loss from the sand would be greater owing to increased transpiration and evaporation.

Methods of Growing the Seedlings

The seeds were sown on 20 March 1950, in clean, steam-sterilized sand in flats. The sand was kept moist until the seedlings were established and a nutrient solution was supplied to them every third day beginning 27 March 1950. This general nutrient solution (see Table II) was continued until 6 April 1950, on which date the seedlings were transplanted into the individual crocks and feeding with the nutrient solutions was started.

The Nutrient Solutions

TABLE X

Parts per million of elements in nutrient solutions

	Levels				
Element	1	2	3	Element	p.p.m.
Nitrogen	208.7	416.3	626.0	Iron	5.00
Phosphorus	40.7	122.0		Zinc	0.05
Potassium	154.7	464.0		Copper	0.02
Magnesium	96.0			Chromium	0.01
Calcium	400.0			Cobalt	0.01
Sulphur	127.0			Molybdenum	0.01
Manganese	0.5			Tungsten	0.01
Boron	0.5				

The chemicals used to supply the major elements for the nutrient solutions were: KH_2PO_4, KNO_3 and NH_4NO_3.

The minor and micro-elements were supplied in the following forms:

Magnesium	- $MgSO_4.7H_2O$	Copper	- $CuSO_4.5H_2O$
Calcium	- $CaCl_2.2H_2O$	Chromium	- $K_2Cr_2O_7$
Manganese	- $MnSO_4.4H_2O$	Cobalt	- $CoCl_2.\ 6H_2O$
Boron	- H_3BO_3	Molybdenum	- $Na_2MoO_4.2H_2O$
Iron	- $Fe(C_6H_5O_7).3H_2O$	Tungsten	- $Na_2WO_4.2H_2O$

Methods of Feeding Plants

The nutrient solutions were supplied in a manner similar to that outlined previously.

It was found that the amount of solution required for the larger crock was 250 ml. This amount of solution was sufficient to completely saturate the soil in the crock and to allow some slight drainage.

Throughout this experiment the plants were fed every five days and the sand was flushed with water before each feeding. Owing to increased evaporation, transpiration, and better drainage it was found necessary to supply small amounts of water to the sand between feedings.

Control of Algae

No trouble was encountered in this experiment with algal growth as the frequent flushings were sufficient to rid the

sand of any algae that appeared. It was found necessary to stir the surface of the sand while flushing to prevent the algae from adhering to the fine sand. No algae developed on the sides of the glazed pots. It should be noted however that the time during which the plants remained in the pots was much shorter than in the previous experiment.

Growth of the Plants

Plant growth during this period was much more rapid than in the earlier period. This was due to the more favourable environmental conditions of sunlight and temperature during these spring months.

The only noticeable difference in the conditions of the plants was the lighter green colour of the plants receiving the lowest levels of all three major elements. This difference was not as great as in the earlier experiment.

The more favourable growing conditions produced a more rapid growth of the plants so that they were ready for harvesting and the determinations were made on 24 May 1950.

The Ascorbic Acid Determinations

The determinations were made in the same manner as those for the winter experiment.

The machine used for these determinations was a Lumetron-photoelectric Colorimeter Model 400 - A. This apparatus used filters to separate the light into monochromatic light which

was allowed to pass through the solution on which the reading was desired. For these tests the filter allowing passage of light with a wavelength of 530 mμ was used.

The colourimeter was adjusted to read 100% transmission when a water blank was placed in the path of the light. This adjustment was checked with the water blank before each reading throughout the determinations. Since the machine was used in series with a voltage stabilizer (constant voltage transformer), drifting of the galvanometer needle was negligible.

Calibration Curve

A calibration curve was prepared using fresh dye, ascorbic acid and 1% metaphosphoric acid solutions. The concentrations of the solutions and the technique were similar to those outlined in the first experiment, with the exception that the machine for this work was set at 100% transmission.

The readings and the calibration curve for the spring experiment are found in the appendix (Figure IIa and Table VIIIa).

Extraction of the Ascorbic Acid

A 25-gram sample of fresh tissue was taken as previously and blended with 200 ml. of the 1% metaphosphoric acid in the Waring blender for three minutes and suction-filtered.

A 10 ml. aliquot of the filtrate was diluted to 50 ml. with metaphosphoric acid and 5 ml. of this final dilution was used with 5 ml. of the dye solution for the reading.

The Determinations

The dye solution used was prepared as before using 20 mg. of the sodium salt of 2-6 dichlorophenol indol phenol.

The 530 mμ filter was used in the colourimeter.

A record of the determinations and the conversions to mg. of ascorbic acid per 100 gm. of fresh tissue is given in the appendix. The calibration curve (Figure IIa) was used for the conversions.

Record and Analysis

The results (Table VIIIa appendix) from the two plants in each treatment were averaged and the experiment was analysed as a factorial with three replications.

The analysis of variance (see appendix Table IXa) shows that for this experiment none of the main effects caused a significant variation in the ascorbic acid concentration of the plants.

TABLE XI

Extract from analysis of variance[1]

Source of variation	F value	F (P .05)
Nitrogen	2.51	3.44
Linear effect of N.	4.96*	4.30
Phosphorus	-	-
Potassium	2.54	4.30

[1]See appendix
*Significant

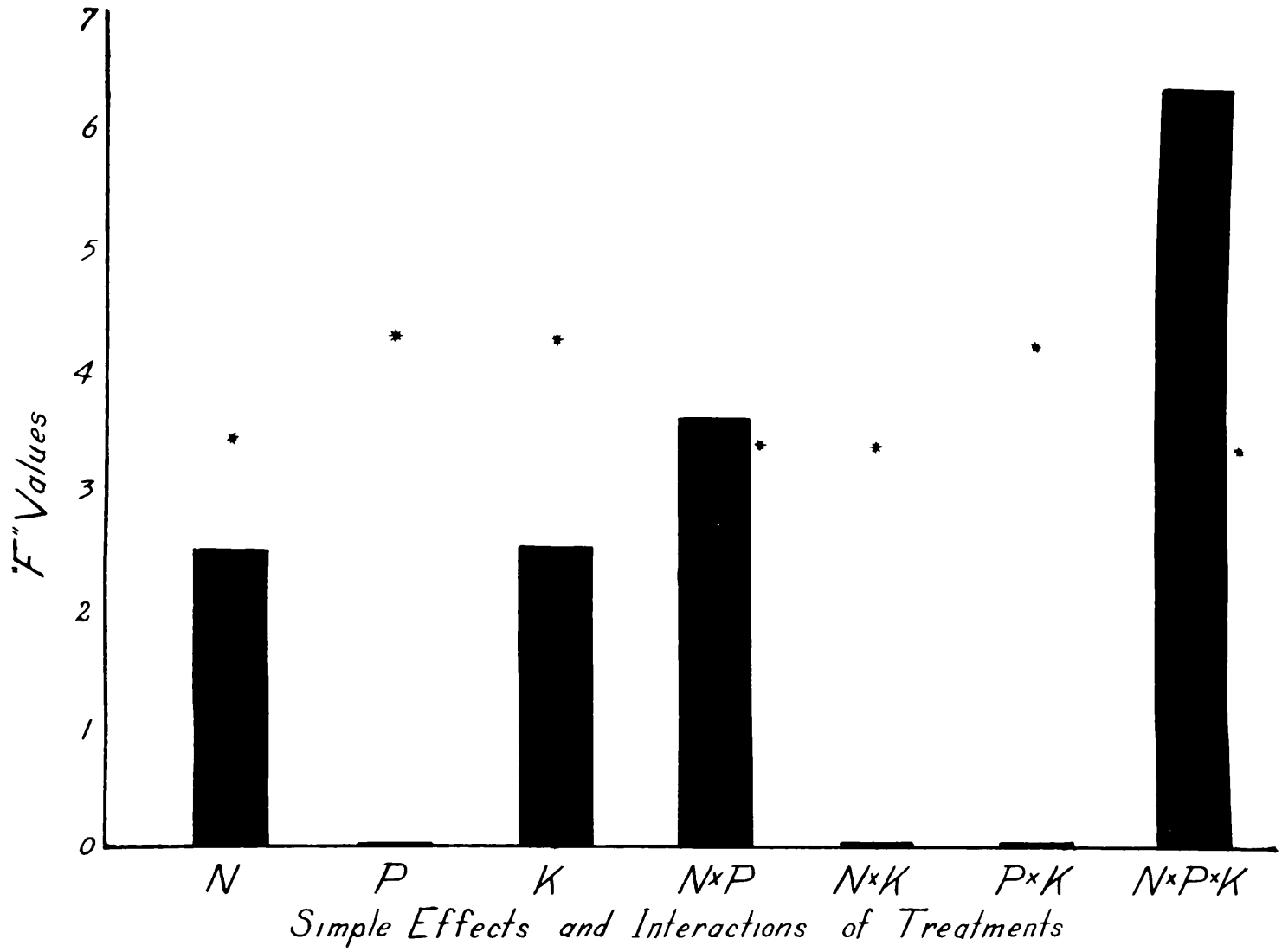


FIGURE II

Spring Experiment

* p .05 level of significance

TABLE XII

Comparison of the ascorbic acid concentration
(Totals of three replications)

Levels	N[1]	P[2]	K[2]
Low	347.42	522.90	541.40
Medium	357.13		
High	363.85	535.50	527.00

[1]Figures represent total for 12 plants.

[2]Figures represent total for 18 plants.

The effect of nitrogen in this experiment was not found to be significant; however the effect that nitrogen did have on the concentration of the vitamin was linear and positive. This followed the same trend as in the previous experiment but it will be recalled that in those tests nitrogen gave a highly significant positive effect.

Phosphorus was found to give a non-significant increase and potassium gave a non-significant decrease in the ascorbic acid concentration. These results are similar to those obtained in the first experiment.

The variation due to treatments was significant at the P .05 level.

The Interaction Nitrogen X Phosphorus

TABLE XIII

Summary of results N X P (Totals)

	1K + 2K	
	1P	2P
1N	178.06	169.36
2N	178.54	178.59
3N	176.30	185.55

From Table XIII it appeared that when the low level of phosphorus was used no increase was obtained in the ascorbic acid concentration by increasing the nitrogen; whereas an increase was caused by increasing the nitrogen when it was in combination with the high level of phosphorus.

The Interaction N X P X K

TABLE XIV

Summary of results N X P X K (Totals)[1]

	1N		2N		3N	
	1K	2K	1K	2K	1K	2K
1P	85.95	92.11	90.57	87.97	92.92	83.38
2P	88.06	81.30	92.06	86.53	91.84	95.71

[1] N.D. for treatment totals at P .05 = 7.2

From Table XIV, the maximum production of ascorbic acid took place in those plants receiving the highest levels of all three major elements.

Comparison of Results of Experiments I and II

The results of both experiments followed a similar pattern. Increases in ascorbic acid concentration were obtained by increasing the level of nitrogen and phosphorus while an increase in potassium gave a decrease in the vitamin C content of lettuce.

In only one instance was a significant "F" value obtained for a main effect. In the winter experiment a highly significant effect of nitrogen was found. The effect of nitrogen on the concentration of the acid was not found to be significant in the spring experiment and it was felt that this was possibly due to the effect of the different environmental conditions which existed during the two periods of growth.

Conclusions

It would appear from these studies that mineral nutrition had some effect upon the ascorbic acid concentration in the leaf tissues of lettuce, however this effect was small and would not account for the variation referred to by Thomas, M. (1947). It was apparent that some other factor or factors besides nutrition must have accounted for the greater part of this variation.

From the results of this work it may be concluded that of the three major elements nitrogen, phosphorus and potassium; nitrogen was most effective in causing variation in the vitamin C content of the plant. The fact that this element gave highly significant variation in one experiment and non-significant results in the other must be attributed to the different environmental conditions under which the tests were carried out in the greenhouses. It is evident that valuable information might be obtained by repeating these experiments when a control of the light factor is possible.

In general the effect of increased nitrogen and phosphorus was to increase the ascorbic acid while potassium has a negative effect upon the content of the acid.

The balance between the levels of the three elements was as important as the actual amount of any one nutrient. This is particularly true of phosphorus and potassium.

The recommendations for the growing of lettuce as given by Thompson, H.C. (1949) should produce a crop of lettuce relatively high in ascorbic acid.

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APPENDIX

Preparation of Nutrient Solutions

The basic nutrient solution used in these experiments was a modification of the solution used by Hill, H. (1940) in his work on plant nutrition.

The chief advantage in using this solution was the fact that the three major elements; nitrogen, phosphorus and potassium were supplied by independent compounds and the concentrations of these elements could be manipulated without causing variation in the concentration of the minor or micro-elements.

Stock solutions were prepared from suitable water-soluble compounds of the required elements.

TABLE Ia

Concentration of Stock Solutions

Compound	Concentration	Compound	Concentration
KH_2PO_4	5% water soln.	$Fe(C_6H_5O_7).3H_2O$	0.05% in water
KNO_3	5% " "	$ZnSO_4.7H_2O$	0.05% "
NH_4NO_3	5% " "	$CuSO_4.5H_2O$	0.05% "
$MgSO_4.7H_2O$	5% " "	$Na_2MoO_4.2H_2O$	151.4 mg. in 4000 ml. N/10 H_2SO_4
$CaCl_2.2H_2O$	5% " "	$K_2Cr_2O_7$	144.0 mg. in 4000 ml. N/10 H_2SO_4
$MnSO_4.4H_2O$	0.05% " "	$Na_2WO_4.2H_2O$	107.7 " "
H_3BO_3	0.05% " "	$CoCl_2.6H_2O$	242.2 mg. in 4000 ml. N/10 H_2SO_4

TABLE IIa

Concentrations of Elements in the 12 Nutrient Solutions
(p.p.m.)

Treatment	N.	P.	K.	Conc. constant in all Nutrient Solutions
3 - 2 - 2	626.0	122.0	464.0	Calcium 400.00
3 - 2 - 1	626.0	122.0	154.7	Magnesium 96.00
3 - 1 - 2	626.0	40.7	464.0	Boron 0.50
3 - 1 - 1	626.0	40.7	154.7	Iron 0.50
2 - 2 - 2	416.3	122.0	464.0	Manganese 0.50
2 - 2 - 1	416.3	122.0	154.7	Zinc 0.05
2 - 1 - 2	416.3	40.7	464.0	Copper 0.02
2 - 1 - 1	416.3	40.7	154.7	Cobalt 0.01
1 - 2 - 2	208.7	122.0	464.0	Chromium 0.01
1 - 2 - 1	208.7	122.0	154.7	Molybdenum ... 0.01
1 - 1 - 2	208.7	40.7	464.0	Tungsten 0.01
1 - 1 - 1	208.7	40.7	154.7	

Methods Used in the Calculation of the Volume of Each Stock Solution Required for the Nutrient Solutions

The following calculations were made for the spring experiment where each treatment was supplied to six plants and each plant received 250 ml. of its nutrient solution. Thus a total volume of 1500 ml. of each nutrient solution was

required. The method for each nutrient solution is the same therefore it is only necessary to include the calculations for the 3 - 2 - 2 solution.

Calculations for the winter experiment were similar, but only 600 ml. of each nutrient solution were required.

Since all the phosphorus was supplied by one compound it was found convenient to begin with this element.

Phosphorus

Required conc. of P. in 3 - 2 - 2 solution ..122.00 p.p.m.

Wt. of P. required in 1,000,000 ml. " ....122.00 gm.

Wt. of P. required in 1,500 ml. " .... 0.183 gm.

Molecular wt. of KH_2PO_4.....136.09 gm.

Atomic wt. of phosphorus 30.98

Wt. of KH_2PO_4 required for 1,500 ml. of solution

$$\frac{136.09 \times 0.183}{30.98} = 0.803 \text{ gm.}$$

Concentration of stock solution 5%

Volume of stock solution required ...$\frac{0.803 \times 100}{5}$ = 16.06 ml.

Therefore 16.06 ml. of the 5% stock solution of KH_2PO_4 were required to supply the required concentration of the phosphorus ion in the 3 - 2 - 2 nutrient solution.

Potassium

Required conc. of K. in the

3 - 2 - 2 solution .....464.00 p.p.m.

Atomic wt. of K. 39.1

Wt. of K. supplied in the KH_2PO_4 (above)

$$\frac{39.1 \times 0.803 \times 1{,}000{,}000}{136.09 \times 1{,}500} = 153.8 \text{ gm.}$$

Wt. of K. to be supplied by KNO_3.....310.2 gm.

Wt. of K. in form of KNO_3 to be supplied in 1,500 ml. of nutrient solution $\frac{310.2 \times 1{,}500}{1{,}000{,}000} = 0.4653$ gm.

Molecular wt. of KNO_3 .....101.1

Wt. of KNO_3 required to supply 0.4653 gm. of K.

$$\frac{101.1 \times 0.4653}{39.1} = 1.20 \text{ gm.}$$

Volume of 5% stock solution required 1.20 x 20 = 24.00 ml.

Nitrogen

Atomic wt. of nitrogen 14.008

Conc. of N. required in nutrient solution .....626 p.p.m.

Wt. of N. supplied in form of KNO_3 .....110.85 gm. p.m.

Wt. of N. to be supplied by NH_4NO_3 .....515.15 gm.

Wt. of N. in form of NH_4NO_3 required in 1,500 ml. of solution = 0.7727 gm.

Wt. of N. in 1 mole. of NH_4NO_3.....28.02 gm.

Molecular weight of " .....80.05 gm.

Wt. of NH_4NO_3 required to supply 0.7727 gm. of N. = 2.207 gm.

Vol. of 5% stock solution required .....44 ml.

Magnesium

The concentration of Magnesium was the same in all nutrient solutions.

Required concentration .....96 p.p.m.

Wt. of mg. required in 1,500 ml. of nutrient soln...0.144 gm.

Molecular wt. of $MgSO_4.7H_2O$.....246.49

Atomic wt. of Mg. 24.32

Wt. of $MgSO_4.7H_2O$required ..... 1.46 gm.

Volume of 5% stock solution required ..... 29.20 ml.

Other Minor Elements

Calculations for boron, calcium, copper, iron, manganese and zinc were similar to those for magnesium.

Element	Compound	Concentration of stock	Vol.of stock required
Ca	$CaCl_2.2H_2O$	5%	44 ml.
B	H_3BO_3	0.05%	8.8 ml.
Fe	$Fe(C_6H_5O_7).\ 3H_2O$	0.05%	80.2 ml.
Mn	$MnSO_4.4H_2O$	0.05%	22.2 ml.
Zn	$ZnSO_4.7H_2O$	0.05%	0.8 ml.
Cu	$CuSO_4.5H_2O$	0.02%	0.24 ml.

Micro-elements

The micro-elements were prepared according to methods advised by Arnon, D.L. (1938).

The compounds were dissolved in N/10 H_2SO_4 and then combined by adding them to N/10 H_2SO_4 to prevent precipitation. The stock solution was then made up to a volume of 4000 ml. with N/10 sulphuric acid.

The calculations for each of these elements were similar. It was found necessary to calculate the weight of the element required and then to find the weight of the compound necessary to supply this weight of the element.

Required final concentration w as 0.01 p.p.m.

Dilution 4000 x 1,500 = 6,000,000

Weight of each element required $\frac{0.01 \times 6{,}000{,}000}{1{,}000{,}000}$ = 60 mg.

Therefore 60 mg. of each element is required in the 4,000 ml. of stock solution.

Weight of compound required = $\frac{\text{Mol. wt.} \times 60 \text{ mg.}}{\text{Atomic wt. x No. of atoms in mol.}}$

Weight of $Na_2MoO_4.2H_2O$ = $\frac{242.03 \times 60 \text{ mg.}}{95.95 \times 1}$ = 151.4 mg.

Weight of $K_2Cr_2O_7$ = $\frac{294.21 \times 60 \text{ mg.}}{52.01 \times 2}$ = 144.0 mg.

Weight of $Na_2WO_4.2H_2O$ = $\frac{329.95 \times 60 \text{ mg.}}{183.92 \times 1}$ = 107.7 "

Weight of $CoCl_2.6H_2O$ = $\frac{237.95 \times 60}{58.94 \times 1}$ = 242.2 "

The volume of this composite micro-element stock solution required for the 1,500 ml. nutrient solution was 1 ml.

Methods of Making up Nutrient Solutions

(a) 1,000 ml. water was placed in the nutrient solution bottle.

(b) Required volumes of major elements, ferric citrate and calcium chloride solutions were added.

(c) All other minor elements were combined previously and added at once. This was found to save time and labour.

(d) 1 ml. of the micro-element stock solution was added.

(e) The volume was made up to 1,500 ml. with water.

Calcium chloride in the presence of the sulphate ion precipitates out and iron becomes unavailable in the presence of other ions therefore these two elements were not included when the minor elements were combined but added to the more dilute nutrient solution separately.

CALIBRATING THE COLOURIMETER

Winter Experiment

Table IIIa records the colourimeter readings obtained when different concentrations of ascorbic acid were used with 5 ml. of the dye solution. Cuvette No. 1, which contained 5 ml. dye, 5 ml. of 1% metaphosphoric acid and zero ascorbic acid was used as a blank and the reading obtained with it (45% transmission) was subtracted from the other readings as in the table. Therefore column 7 of the table represents the percent transmission due to the reduction of dye by the ascorbic acid.

Column 5 of Table IIIa gives the concentration of ascorbic acid in each cuvette in μgm. per ml. The calculations for this column are explained below.

TABLE IIIa

Calibration Readings[1]

1 No.	2 Volume dye soln.	3 Volume 1% meta-phos. acid	4 Volume ascorbic acid	5 Ascorbic acid μgm./ml.	6 Trans-mittance %	7 Trans-mittance due to ascorbic acid
1	5 ml.	5 ml.	0 ml.	0	45.0	-
2	5	4.0	1.0	1.0	52.0	7.0
3	5	3.5	1.5	1.5	56.0	11.0
4	5	3.0	2.0	2.0	59.5	14.5
5	5	2.5	2.5	2.5	64.0	19.0
6	5	2.0	3.0	3.0	68.5	23.5
7	5	1.5	3.5	3.5	71.5	26.5
8	5	1.0	4.0	4.0	74.0	29.0
9	5	0.5	4.5	4.5	74.0	29.0
10	5	0	5.0	5.0	75.0	30.0

[1] The total volume in each cuvette was 10 ml.

To Calculate Column 5 in Table IIIa

Conc. of stock solution of ascorbic acid ..100 mg./100 ml. 1% metaphosphoric acid

Conc. of diluted solution of ascorbic acid 1 ml. stock/ 100 ml. 1% metaphosphoric acid

Wt. of ascorbic acid in 1 ml. of stock ..... 1 mg.

Wt. of ascorbic acid in 1 ml. of dilution.....1/100 mg.

= 10μgm.

Therefore for each 1 ml. of diluted solution put in the cuvette there was 10 gm. of ascorbic acid added. Since there were 10 ml. of solution in the cuvette there was 1μgm. of ascorbic acid per ml. for each 1 ml. of diluted ascorbic acid solution added.

The Calibration Curve

Columns 5 and 7 of Table IIIa were used as axes in preparing the calibration curve (Figure Ia).

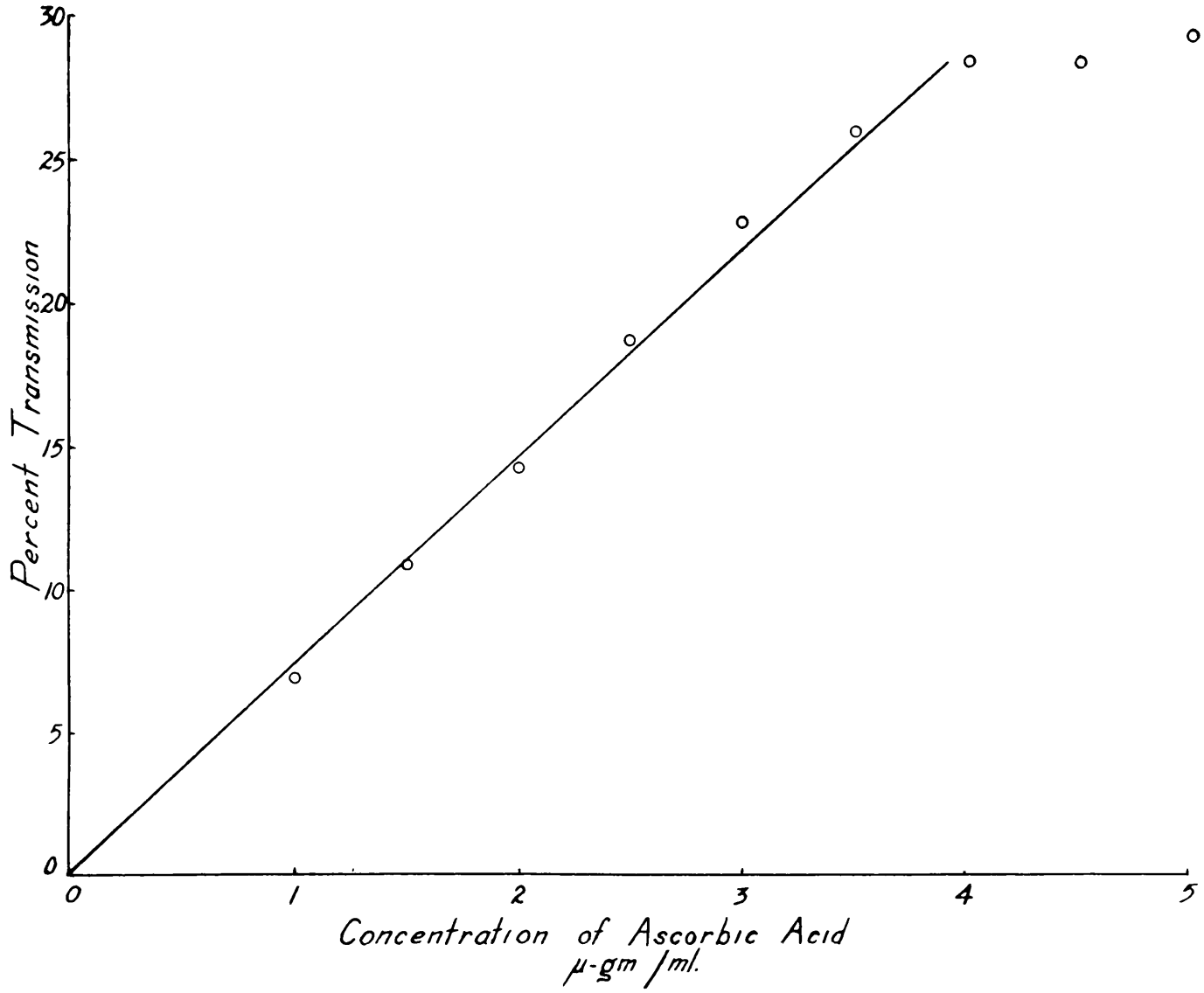

FIGURE Ia

Calibration Curve for Winter Experiment

Table IV a.

The Record of Results of Determinations
(Winter Experiment)

Replication I.

1 Treatment	2 % Transmission	3 % Trans. due to ascorbic acid (2) - 45%.	4[1] μ gm/ml cal.curve. "y"	5[2] mg. Ascorbic Acid/100gm. fresh tissue (y) X 9
3 - 2 - 2	69.0%	24.0%	3.22	28.98
3 - 2 - 1	68.5	23.5	3.17	28.53
3 - 1 - 2	68.7	23.7	3.20	28.80
3 - 1 - 1	70.5	25.5	3.43	30.87
2 - 2 - 2	69.5	24.5	3.30	29.70
2 - 2 - 1	68.7	23.7	3.20	28.80
2 - 1 - 2	66.5	21.5	2.92	26.28
2 - 1 - 1	70.0	25.0	3.37	29.43
1 - 2 - 2	71.0	26.0	3.50	31.50
1 - 2 - 1	67.8	22.8	3.07	27.63
1 - 1 - 2	61.0	16.0	2.17	19.53
1 - 1 - 1	69.5	24.5	3.30	29.70

Replication II

3 - 2 - 2	66.5	21.5	2.92	26.28
3 - 2 - 1	72.5	27.5	3.70	33.30
3 - 1 - 2	71.0	26.0	3.50	31.50
3 - 1 - 1	72.0	27.0	3.64	32.76
2 - 2 - 2	73.5	28.5	3.83	30.47
2 - 2 - 1	70.5	25.5	3.43	30.87
2 - 1 - 2	68.0	23.0	3.10	27.90
2 - 1 - 1	67.5	22.5	3.04	27.36
1 - 2 - 2	69.0	24.0	3.22	28.98
1 - 2 - 1	64.0	19.0	2.57	23.13
1 - 1 - 2	63.0	18.0	2.43	21.87
1 - 1 - 1	70.0	25.0	3.37	30.33

1 The 45% transmission was obtained with the blank.

2 The 9 is conversion factor, the calculation which is given hereunder.

TABLE IV a (cont.)

Replication III

1	2	3	4	5
3 - 2 - 2	72.0	27.0	3.64	32.76
3 - 2 - 1	66.0	21.0	2.85	25.65
3 - 1 - 2	66.5	21.5	2.90	26.10
3 - 1 - 1	71.5	26.5	3.57	32.13
2 - 2 - 2	71.5	26.5	3.57	32.13
2 - 2 - 1	69.5	24.5	3.30	29.70
2 - 1 - 2	68.5	23.5	3.17	28.53
2 - 1 - 1	69.5	24.5	3.30	29.70
1 - 2 - 2	68.0	23.0	3.11	27.99
1 - 2 - 1	66.0	21.0	2.85	25.65
1 - 1 - 2	63.5	18.5	2.50	22.50
1 - 1 - 1	68.5	23.5	3.17	28.53

TABLE V a

Compilation of Table IVa

Ascorbic Acid Content mg. per 100 gms. fresh tissue.

Treatment	Rep. I	Rep. II	Rep. III	Total
3 - 2 - 2	28.98	26.28	32.76	88.02
3 - 2 - 1	28.53	33.30	25.65	87.48
3 - 1 - 2	28.80	31.50	26.10	86.40
3 - 1 - 1	30.87	32.76	32.13	95.76
2 - 2 - 2	29.70	30.47	32.13	92.30
2 - 2 - 1	28.80	30.87	29.70	89.37
2 - 1 - 2	26.28	27.90	28.53	82.71
2 - 1 - 1	29.43	27.36	29.70	86.49
1 - 2 - 2	31.50	28.98	27.99	88.47
1 - 2 - 1	27.63	23.13	25.65	76.41
1 - 1 - 2	19.53	21.87	22.50	63.90
1 - 1 - 1	29.70	30.33	28.53	88.56
Totals	339.75	344.75	341.37	1025.87

The Conversion Factor

This factor served to convert the reading from the calibration curve to mg. of ascorbic acid per 100 gm. of fresh plant tissue.

To arrive at this factor it was necessary to work through the various dilutions, etc., made during the determinations.

Calculating the conversion factor.

Let "y" μgm./ml. represent the concentration of ascorbic acid in the cuvette as read from the calibration curve.

Wt. of sample of fresh tissue ..... 25 gm.

To bring to 100 gm. of tissue ..... x 4

Wt. of solution in blendor 25 gm. tissue,

200 gm. solution .....225 gm.

Amt. of blended solution used ... 10 ml. $\times \frac{225}{10}$

The 10 ml. were diluted to 50 ml. and 5 ml.

were used $\times \frac{50}{5}$

Volume in cuvette .....10 ml. $\times \frac{10}{1}$

To convert μgm. to mgs. ..... $\times \frac{1}{1000}$

Therefore conversion factor $= y \times \frac{1}{1000} \times 10 \times \frac{50}{5} \times \frac{225}{10} \times 4$

$= 9y.$

TABLE VIa

Analysis of Variance (Concentration of Ascorbic Acid)

Winter Experiment

Source	D.F.	S.S.	M.S.	F.	F(P.05)	F(P.01)
Reps.	2	1.08	0.54			
Treatments	11	251.22	22.84	4.98**	2.27	3.19
Nitrogen	2	77.66	38.83	8.46**	3.44	5.72
L[1]	1	67.74	67.74	14.77**	4.30	7.94
Q	1	9.93	9.93	2.16		
Phosphorus	1	9.22	9.22	2.10		
Potassium	1	13.69	13.69	2.98		
N X P	2	20.29	10.15	2.21		
N X P	2	6.09	3.05	0.64		
P X K	1	79.10	79.10	17.25	4.30	7.94
N X P X K	2	45.09	22.55	4.92*	3.44	
ERROR	22	100.90	4.586			
Total	35	353.20				

**Indicates significance at the P .01 level.

*Indicates significance at the P .05 level.

[1]L. represents the linear effect of the nitrogen treatment.
Q. represents the quadratic effect of the nitrogen treatment.

Calculation of Sums of Squares (Winter Experiment)

Legend: S.S. = Sums of squares

S. = Summation

S.S. for Total

$$= \frac{S(28.98^2 + \ldots\ldots\ldots\ldots 28.53^2)}{1} - \frac{1025.87^2}{36} = 353.20$$

S.S. for Replications

$$= \frac{S(339.75^2 + 344.75^2 + 341.37^2)}{12} - \frac{1025.87^2}{36} = 1.08$$

S.S. for Treatments

$$= \frac{S(88.02^2 + \ldots\ldots\ldots\ldots 88.56^2)}{3} - \frac{1025.87^2}{36} = 251.22$$

S.S. for Nitrogen

$$= \frac{S(317.34^2 + 350.87^2 + 357.66^2)}{12} - \frac{1025.87^2}{36} = 77.66$$

S.S. for Linear Effect of Nitrogen (k = 12)

$$= \frac{(T_{3N} - T_{1N})^2}{2k} = 67.74$$

S.S. for Quadratic Effect of Nitrogen

$$= \frac{(2T_{2N} - T_{1N} - T_{3N})^2}{6k} = 9.93$$

S.S. for Phosphorus

$$= \frac{S(503.82^2 + 522.65^2)}{18} - \frac{1025.87^2}{36} = 9.22$$

S.S. for Potassium

$$= \frac{S(524.07^2 + 501.80^2)}{18} - \frac{1025.87^2}{36} = 13.69$$

S.S. for Interaction N X P

	1K + 2K	
	1P	2P
1N	152.45	164.88
2N	169.20	181.67
3N	182.66	175.50

$$= \frac{S(152.45^2 + \ldots 175.50^2)}{6} - \frac{1025.87^2}{36} - (\text{S.S.for N} + \text{S.S.for P})$$

$$= 20.29$$

S.S. for remaining interactions were calculated as above.

To Calculate Necessary Difference (Totals)

N.D. $= \sqrt{\text{M.S. Error} \times 2 \times n} \times$ "t" value for D.F. (Error)

N.D. for N (Totals P.05) $= \sqrt{4.586 \times 2 \times 12} \times 2.07$

$= 23.07$

N.D. for N (Totals P.01) $= \sqrt{4.586 \times 2 \times 12} \times 2.82$

$= 29.58$

N.D. for Treatment (Totals P.05) $= \sqrt{4.586 \times 2 \times 3} \times 2.07$

$= 10.84$

N.D. for Treatment (Totals P.01) $= \sqrt{4.586 \times 2 \times 3} \times 2.82$

$= 14.77$

SPRING EXPERIMENT

Calibration Curve

The Lumetron photoelectric colourimeter Model 400A was calibrated as the machine in the previous experiment. A setting of 100% transmission was used with the water blank.

TABLE VIIa

Calibration Readings (Spring Experiment)[1]

1 Cuvette	2 µgm. Ascorbic acid per ml. in cuvette	3 % Transmission reading	4 % Transmission[2] due to ascorbic acid
1	0.0	62.00	0.00
2	1.0	69.50	7.50
3	1.5	74.00	12.00
4	2.0	79.00	17.00
5	2.5	84.00	22.00
6	3.0	89.00	27.00
7	3.5	93.50	31.50
8	4.0	93.50	31.50

1) 5 ml. of dye solution of same concentration was used as in the winter experiment. The total volume in each cuvette was 10 ml.

2) Figures in this column were obtained by subtracting 62% transmission (blank) from readings in column 3.

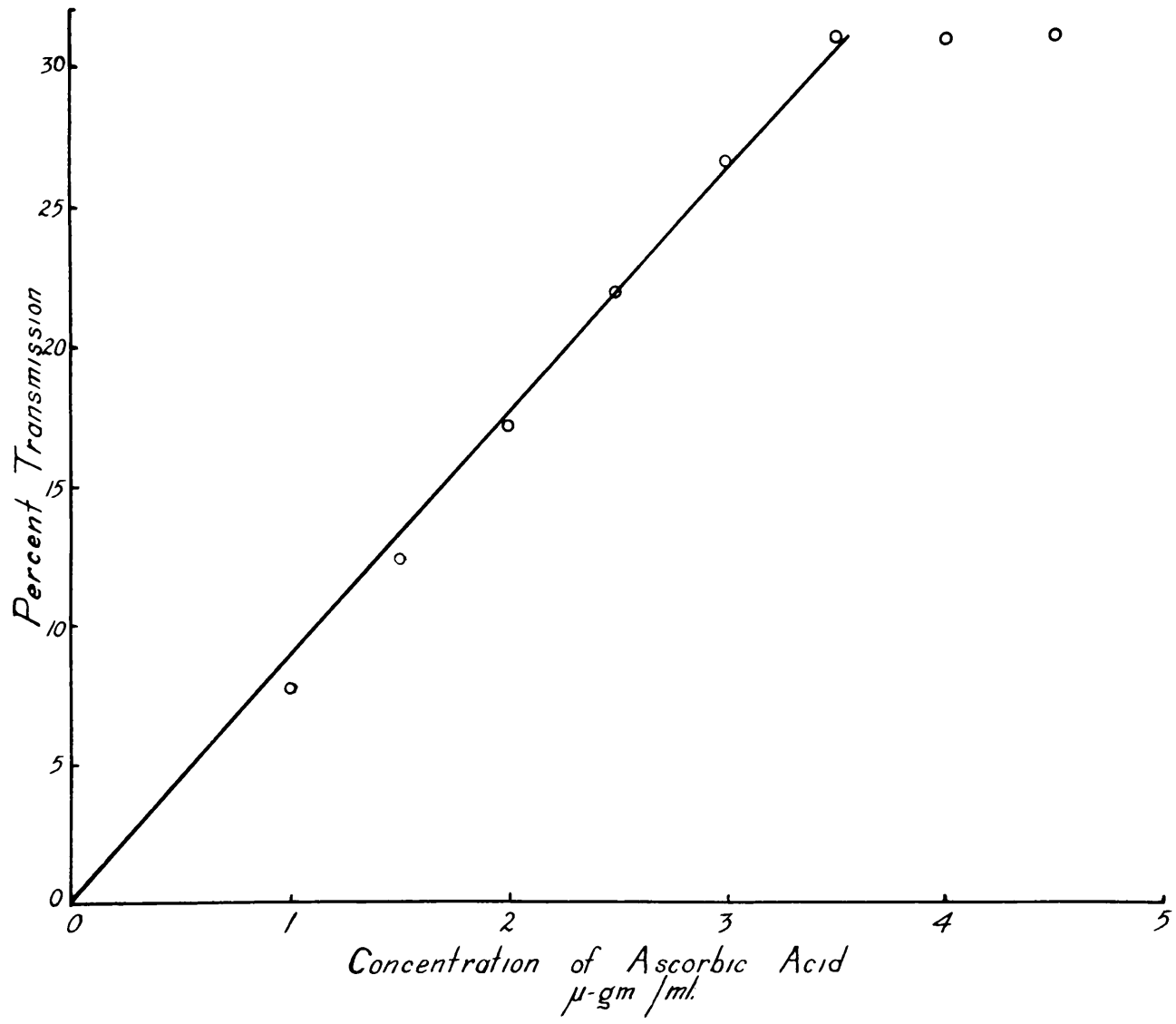

FIGURE IIa

Calibration Curve for Spring Experiment

TABLE VIIIa

Record of Ascorbic Acid Concentration (Spring Experiment)

Treatment	Plant 1	Plant 2	Average for Treatment mg./100 gm. tissue
Replication I			
3 - 2 - 2	31.41	31.41	31.41
3 - 2 - 1	30.33	30.87	30.60
3 - 1 - 2	27.90	28.35	28.12
3 - 1 - 1	30.33	28.89	29.61
2 - 2 - 2	28.35	29.34	28.84
2 - 2 - 1	29.34	32.40	30.87
2 - 1 - 2	23.04	32.40	27.72
2 - 1 - 1	32.40	31.41	31.90
1 - 2 - 2	27.36	22.05	24.70
1 - 2 - 1	30.33	27.36	28.84
1 - 1 - 2	31.41	32.40	31.90
1 - 1 - 1	24.12	29.34	26.73
Replication II			
3 - 2 - 2	32.40	32.40	32.40
3 - 2 - 1	31.41	31.41	31.41
3 - 1 - 2	23.04	31.41	27.72
3 - 1 - 1	31.41	32.40	31.90
2 - 2 - 2	29.34	29.34	29.34
2 - 2 - 1	30.33	29.34	29.83
2 - 1 - 2	29.34	31.41	30.37
2 - 1 - 1	28.35	25.20	26.77
1 - 2 - 2	29.34	30.33	29.83
1 - 2 - 1	31.41	30.33	30.87
1 - 1 - 2	30.33	31.41	30.87
1 - 1 - 1	31.41	28.35	29.88
Replication III			
3 - 2 - 2	32.40	31.41	31.90
3 - 2 - 1	30.33	29.34	29.83
3 - 1 - 2	25.74	29.34	27.54
3 - 1 - 1	31.41	31.41	31.41
2 - 2 - 2	27.36	29.34	28.35
2 - 2 - 1	30.33	32.40	31.36
2 - 1 - 2	31.41	28.35	29.88
2 - 1 - 1	31.41	32.40	31.90
1 - 2 - 2	25.20	29.34	27.27
1 - 2 - 1	28.35	28.35	28.35
1 - 1 - 2	30.33	28.35	29.34
1 - 1 - 1	30.33	28.35	29.34

Record and Analysis

The determinations were made in a manner similar to that of the previous experiment and calculations were carried out in the same way.

The sums of squares for the analysis of variance were calculated according to the same methods as in winter experiment, using the data presented in Table VIIIa.

TABLE IXa

Analysis of Variance (Concentration of Ascorbic Acid)

(Spring Experiment)

Source	D.F.	S.S.	M.S.	F.	F(P .05)
Reps.	2	4.13	2.065		
Treatments	11	66.06	6.005	2.65*	2.27
Nitrogen	2	11.38	5.69	2.51	3.44
L 1		11.24	11.24	4.96*	4.30
Q 1		0.13			
Phosphorus	1	0.20			
Potassium	1	5.76	5.76	2.54	4.30
N X P	2	16.65	8.32	3.67*	3.44
N X K	2	2.45	1.23		
P X K	1	0.16			
N X P X K	2	29.46	14.73	6.506*	3.44
ERROR	22	49.81	2.264		
Total	35	120.00			

*Indicates significance at the P .05 level.

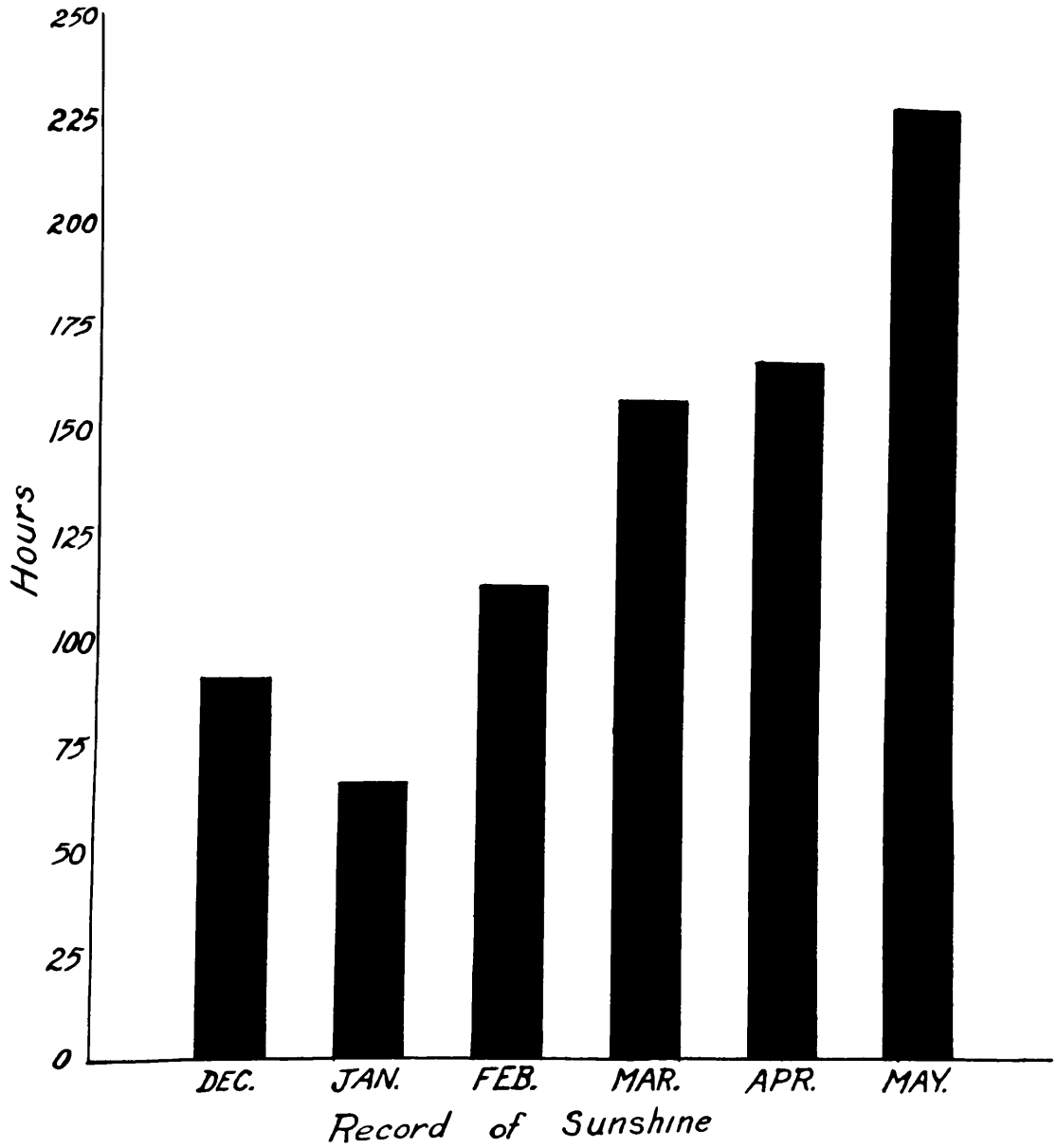

FIGURE IIIa

Beaulieu, Hon. Paul, C.A. (1950)