UTILIZATION OF NITRATES BY THE COFFEE PLANT

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

JUNE 1944

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ACKNOWLEDGMENTS

The author wishes to express his sincerest appreciation to Dr. L. A. Dean, formerly of the Hawaii Agricultural Experiment Station, for making it possible for the author to work on this problem. He is deeply grateful to the Hawaii Agricultural Experiment Station and the Botany Department of the University of Hawaii for the use of facilities and equipment without which the work could not have been done. He also wishes to thank the Hawaiian Sugar Planters' Association for the use of the tubes to measure sunlight intensity.

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INTRODUCTION

In the course of an investigation (19) dealing with the levels of nitrogen, potassium, and carbohydrates in the leaves of coffee trees (*Coffea arabica* L.) grown at Kona, Hawaii, it was found that in comparison with other plants relatively large amounts of nitrate nitrogen were accumulated by young coffee leaves (2-10 months old), and that the nitrate content was found to fluctuate widely during the growing season. No adequate explanation for this seemingly unusual behavior was found. For this reason, it was decided that a study should be made to determine the cause or causes which lead to the accumulation of nitrates in coffee leaves. After a consideration of the important external factors influencing nitrate metabolism, it appeared that at Kona sunlight intensity may be the limiting factor in the process; hence, in this investigation the chief emphasis was placed on the effect of different degrees of sunlight intensity on the assimilation of nitrates by the coffee plant. In order to have a strict control over nutrient supply, the coffee plants were grown in water culture. This paper presents the results of the investigation.

Review of Literature

It is now widely accepted that certain specific external and internal factors play dominant roles in the nitrate nutrition of green plants. An excellent review of these factors has been published by Nightingale (46). It is known that with a plentiful, external supply of nitrate many plants store large amounts of nitrate in their roots, stems, or leaves without injurious effects (2, 12, 17, 42, 71, 78). The nitrate content has been found to vary with different treatments and with the stage of growth (2, 12, 17, 22, 71). The findings of several investigators indicate that nitrate absorption proceeds most favorably in a rather acid medium (1, 14, 32, 69).

When certain conditions prevail within the plant, the inorganic nitrate nitrogen is transformed to protein nitrogen. The exact process of this transformation is still largely a matter of conjecture, but the results of numerous investigations indicate that in the initial phase of the process nitrate is reduced to nitrite, and then to ammonia (20, 28, 46, 53). In the following phase, amino acids are formed through the combination of ammonia and organic acids. A subsequent series of complex reactions and condensations results in the formation of proteins. The organs in which these reactions occur are known to vary for different plants.

The fact that energy from the oxidation of sugars is utilized by the plant in the reduction of nitrates has been definitely established. Thus, whether the process proceeds in light or in darkness, it has been found to be accompanied by an increase in respiration (28) and a decrease in carbohydrates (20, 52, 56, 59, 60, 65, 66, 67, 68). The enzyme reducase has also been shown to be of importance in the reduction process (20, 21, 23, 35). From the foregoing, it is obvious that any condition which affects either the respiratory process or the activity of reducase becomes a factor in the assimilation of nitrates. For example, sunlight through its part in the synthesis of carbohydrates plays a role which can not be overlooked (24, 36, 59, 60). Because of the regulatory effect of temperature upon respiration and possibly also upon the activity of reducase (35, 45), temperature changes can lead to the accumulation or assimilation of nitrates (44, 49, 50, 53). Many papers have been published which have attributed the accumulation of nitrates to a lack of potassium (47, 54, 62), of phosphorus (21, 23, 62), or of manganese (39, 77). These results have been explained to be due to the roles that phosphorus and potassium play in reducase synthesis (21, 23), in carbohydrate metabolism (21, 33, 37), in nitrogen metabolism (47), in respiration (33), and in other processes (31, 33, 70) that affect the well-being of the plant. It has been claimed that calcium and sulfur deficiencies cause decreases in reducase activity (23). A slightly alkaline medium has been shown to be a

requirement for high reducase activity (20). It has been reported that the reduction and assimilation of nitrates are limited by a high concentration of salts in the culture solution (51). Furthermore, plants growing under low soil moisture conditions have been found to accumulate nitrates (25).

It is interesting to note that according to Vickery et al. (73), who worked with excised tobacco leaves which were placed in distilled water under dim light, production of nitrates from organic nitrogen by plants appears to be a possibility. So far, however, no one has confirmed this observation. On the contrary, Nightingale (43) has found that proteolysis to nitrates did not occur in tomato plants even after 284 hours of continuous darkness.

EXPERIMENTAL METHODS

The coffee seeds (Coffea arabica L.) used in this study were obtained from a single tree growing at Kona, Hawaii. About 500 seeds were planted in a seed box. From the germinated seeds, 92 vigorous seedlings were selected as the experimental plants. The seedlings were placed in a very dilute nutrient solution to encourage root growth. At this stage, the plants were kept under shade for three weeks. Throughout the experiment culture solutions were aerated moderately and continuously, and their reactions were maintained between pH 4.5-6.5. On February 10, 1943, the plants were transferred to gallon jars containing the complete nutrient solution which was changed once every three weeks. Two seedlings were placed in each jar. The composition of the complete nutrient solution was as follows:[1]

KNO_3--0.0012 M
$Ca(NO_3)_2$--0.0012 M
KH_2PO_4--0.00025 M
$MgSO_4.7\ H_2O$--0.0012 M
5 cc. of a 2% stock solution of ferric tartrate.
5 cc. from a liter of solution containing 0.25 gm. H_3BO_3, 0.5 gm. $MnCl_2 \cdot 4\ H_2O$, 0.25 gm. $ZnSO_4$, 0.1 gm. $CuSO_4 \cdot 5\ H_2O$.

On February 24, 44 plants were placed in the open; 24 plants were placed under shade no. 1, and the remaining 24 plants were placed under shade no. 2. Shade no. 1 (Fig. 1) was built of laths 2 cm. in width which were spaced 2 cm.

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[1]Preliminary investigations showed that best growth was made in solutions of low osmotic pressure.

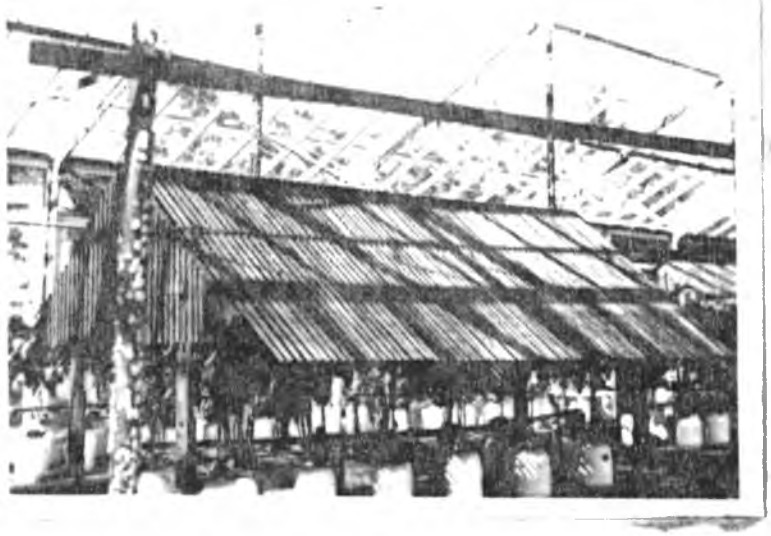

Fig. 1. Shade no. 1. Constructed to give one-half shade.

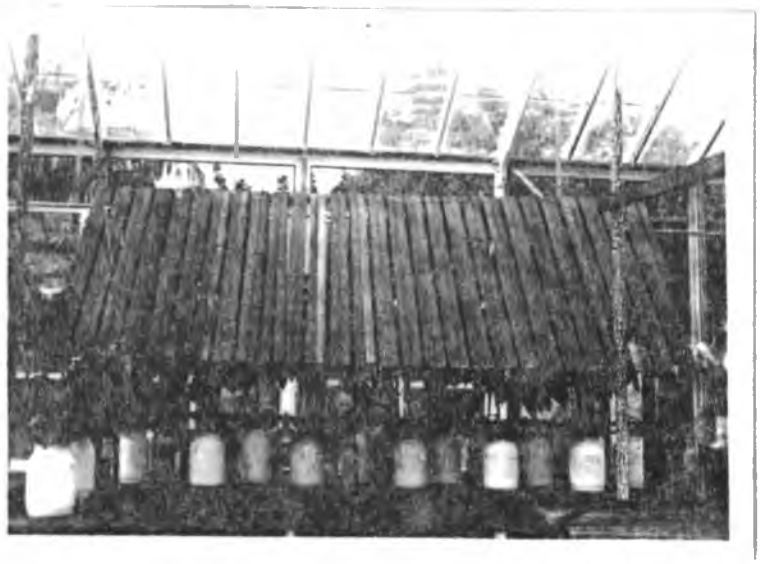

Fig. 2. Shade no. 2. Constructed to give three-fourths shade.

apart to give one-half shade. Shade no. 2 (Fig. 2) was built of 4 cm. laths spaced 2 cm. apart to give two-thirds shade. Three weeks later, in order to increase the shade to three-fourths, the width of the laths of shade no. 2 was increased to 8 cm., while the spacing was kept at 2 cm. The shades were so constructed that practically all of the direct sunlight which fell upon the plants entered through the roofs. Hereafter in this paper shade no. 1 will be referred to as one-half shade, and shade no. 2 as three-fourths shade.

Commencing on February 24, growth measurements and sunlight radiation records were made once a week. Height was measured from the cotyledonary node up to the growing tip of the main stem. Sunlight intensity was measured by the decomposition of oxalic acid in 0.01 M uranyl sulfate solution. The method has been described by Anderson and Robinson (3). It has been shown that the sunlight record obtained outside the greenhouse by this method is comparable with that obtained by the Leeds and Northrup pyrheliometer (9). The oxalic acid-uranyl sulfate solution was placed in an L-shaped, painted test tube with an internal diameter of 22 mm. and an angle of 125°. Around the lower arm of the tube was an unpainted band 6 mm. in width. When exposed to light, the tube was set up so that the unpainted band was always normal to the path of the sun rays.

On June 30, approximately 27 weeks after germination, the coffee plants were divided into six series and the

following treatments were initiated:

Series	No. of plants	Treatments
I A	8	Complete nutrient; no shade
B	6	" " ; one-half shade
C_1	6	" " ; three-fourths shade
II A_1	8	3 wks. before sampling -N; no shade
B_1	6	" " " " "; ½ shade
C_1	6	" " " " "; ¾ "
III A_1	8	3 wks.-N, followed by 3 wks. +N; no shade
B_1	6	" " ", " " " " "; ½ shade
C_2	6	" " ", " " " " "; ¾ "
IV A_2	8	6 wks. before sampling -K; no shade
B_2	6	" " " " "; ½ shade
C_2	6	" " " " "; ¾ "
V[3]	6	6 wks. before sampling -P; no shade
VI	6	6 wks. before sampling +2N; no shade

On August 4, leaves were sampled from all plants; only fully matured leaves were taken. After weighing, the fresh leaves were immediately dried under strong draft in an electric oven at 80-90° C. The dried leaves were ground to 40 mesh, and were analyzed for various nitrogen and carbohydrate fractions by the following methods:

Methods of Analysis

Total nitrogen was determined by the Kjeldahl method after preliminary reduction with reduced iron powder (57).

Soluble nitrogen was extracted from the dried tissues with distilled water. A sample of 2.5-5.0 gm. was placed in a beaker, 200 cc. of water added, and the beaker placed in a water-bath at 80° C. for 10 minutes (74). The

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[1] KNO_3 and $Ca(NO_3)_2$ were replaced by equimolar concentrations of KCl and $CaCl_2$.

[2] KNO_3 and KH_2PO_4 were replaced by equimolar concentrations of $NaNO_3$ and NaH_2PO_4.

[3] KH_2PO_4 was replaced by an equimolar concentration of KCl.

contents were then transferred to a 250 cc. volumetric flask, cooled, and made up to volume. The mixture was filtered through a coarse filter. Total soluble nitrogen was determined in an aliquot of the filtrate.

Insoluble nitrogen was determined by subtracting total soluble nitrogen from the total nitrogen.

Nitrate nitrogen was determined in an aliquot of the filtrate obtained as described in the method for soluble nitrogen. The aliquot was placed in a Kjeldahl flask, and distilled water and ignited MgO added. After distilling over about 200 cc. of water, the distillation was interrupted and the flask cooled to room temperature. Distilled water and one gram of Devarda's alloy were then added, and distillation was resumed. Distillation was continued until 300 cc. of distillate had been distilled into N/28 H_2SO_4. Nitrogen distilled over during the second distillation was determined by back titration with N/28 NaOH.

Ammonia nitrogen was determined by the method of Pucher et al. (58) in an aliquot of the filtrate obtained as described in the determination of soluble nitrogen.

Amide nitrogen was determined in an aliquot of the soluble nitrogen filtrate by the method of Pucher et al. (58).

Alpha-amino nitrogen was determined in the residue from the ammonia determination by the Van Slyke method (40, p. 317).

Peptide nitrogen was determined in an aliquot of the filtrate obtained in the determination of soluble nitrogen.

The acid hydrolysis method of Vickery et al. (72) was used.

Basic nitrogen was determined in an aliquot of the soluble nitrogen filtrate. Phosphotungstic acid was used as the precipitant. Total nitrogen was determined in the precipitate after it had been washed with a dilute solution of phosphotungstic acid.

Soluble sugars were extracted with hot 80% alcohol from 2.0 gm. samples of dried tissue. The extracts were cleared with neutral lead acetate. Sucrose was hydrolyzed with invertase (40, p. 261). The amount of sugar in the extracts was determined by the method of Quisumbing-Thomas (8).

Starch was determined in the residue from the alcohol extract. The residue was boiled, treated with saliva, cleared with neutral lead acetate, and hydrolyzed with acid (40, p. 262).

Hemicellulose was determined in the residue remaining after treatment with saliva. The residue was hydrolyzed with (1 + 20) HCl for three hours (40, p. 262).

Potassium was determined in the ashed tissue by the Na-cobaltinitrite method of Volk and Truog (75). Samples of dried tissue were digested with a nitric-perchloric acid mixture. After ammonia was driven off, the residue was taken up with dilute HCl and made up to volume with distilled water. Suitable aliquots were taken for the determination of potassium.

Calcium was determined volumetrically as the oxalate in an aliquot of the solution obtained in the "wet ash" method

described under the determination of potassium.

Phosphorus was determined colorimetrically as the molybdate in an aliquot of the solution obtained in the "wet ash" method described under the determination of potassium.

DISCUSSION OF RESULTS

Appearance Of Plants

At the conclusion of this investigation, the coffee plants grown under the various light treatments showed pronounced differences in appearance. Photographs of typical plants from each light treatment are shown in Fig. 3.

Contrary to the results obtained by others who investigated the effects of shading on the coffee plant (4, 6, 7), the unshaded plants showed no ill effects due to full sunlight. On the whole, some of the morphological differences arising from differences in shading were similar to those previously reported. Thus, shading increased the length of internodes (4, 5, 7), increased leaf size (4, 5, 7, 26), but decreased the size of the root system (27). Although the leaves of the unshaded plants were smaller and their color was not as dark green as that of the shaded ones, they, nevertheless, appeared to be just as vigorous as the shaded ones. In comparison with the shaded leaves, the leaves of plants grown in full sunlight were characterized by their narrowness. There were more leaves per plant in the unshaded series than in either of the shaded series. The trunks of the unshaded plants were much thicker than those of the shaded plants. Plants grown under three-fourths shade appeared rather spindling, and had the least number of leaves per plant. In most respects, the plants grown under one-half shade occupied an intermediate position between those grown

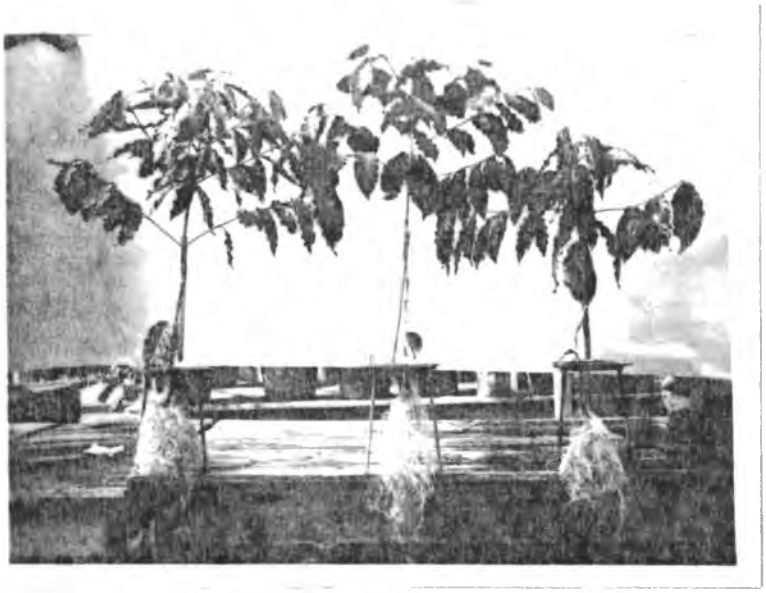


Fig. 3. Coffee plants grown under three different light conditions. Left to right: no shade, one-half shade, three-fourths shade.

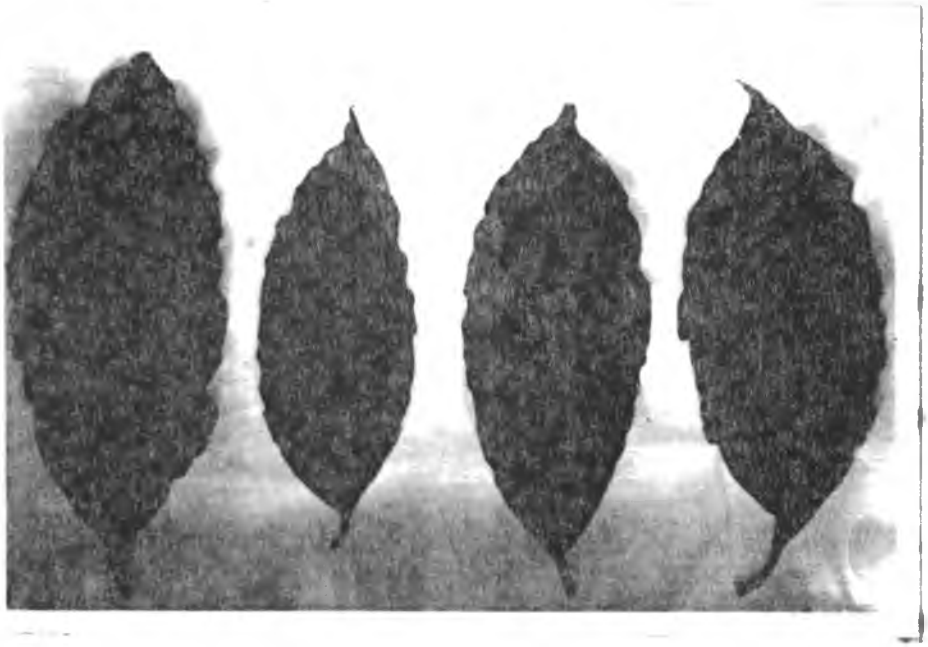


Fig. 4. Coffee leaves showing progressively increasing severity of potassium deficiency (l to r). First leaf on left is a normal leaf.

without shade and those grown under three-fourths shade. It can be said without reservation that the plants in the open and those under one-half shade appeared much hardier than those under three-fourths shade.

In the nitrogen series (series II and III), the unshaded leaves were yellowish green after the end of three weeks of no nitrogen, thus showing the effects of nitrogen deficiency. Corresponding shaded leaves showed no signs of nitrogen starvation. Toward the end of the experiment the minus-potassium plants (series IV) grown without shade developed symptoms of potassium deficiency. Necrotic areas appeared along the margins of mature leaves. As the deficiency progressed, these areas of dead tissues gradually increased in size along the leaf margin. In Fig. 4 are photographs of three leaves showing various stages of potassium deficiency. As in the nitrogen series, shaded plants showed no signs of potassium deficiency. The plants grown without phosphorus (series V) did not show any visible symptoms of its absence.

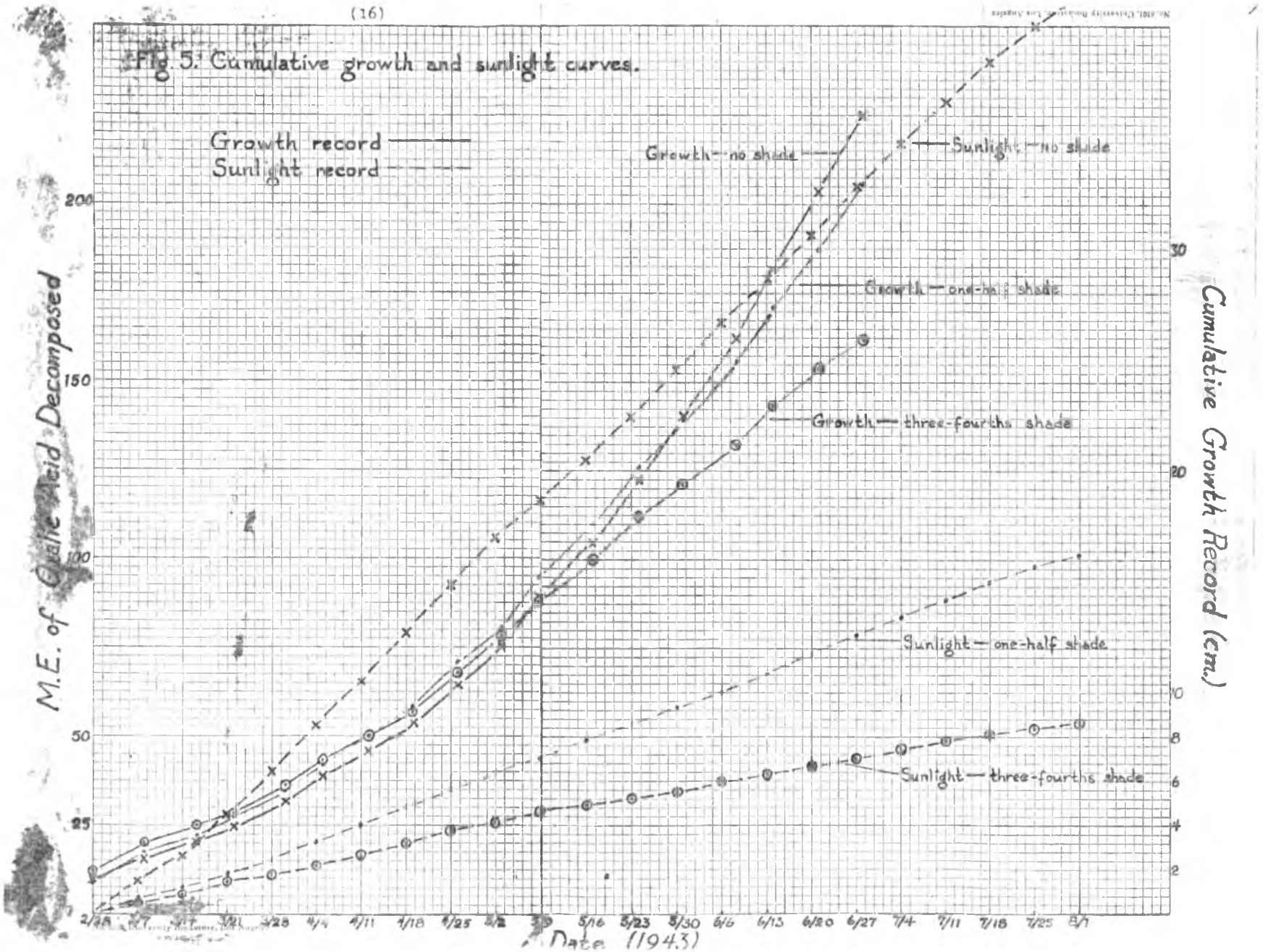
Relationship Between Growth And Sunlight Intensity

In many of the coffee producing countries, the coffee plant is frequently grown under shade. It has long been the general belief that shading is essential for the best growth of coffee plants, especially of the Arabian type. Yet, as far back as 1901, after a canvass of the subject of shade in coffee culture, Cook (16) came to the conclusion that there is no basis for the belief that shade is a necessary condition for the growth of the coffee plant. With some plants such as potato, lettuce, and radish, Shantz (63) found that shading increased growth; while with other plants such as corn, it failed to do so. Popp (55) reported that soybean plants attained their greatest height under medium light intensities. Those plants receiving the greatest amount of light, however, were the most vigorous, produced the best leaves and color, and the best fruit.

The growth of the coffee plants and the sunlight intensity during the duration of this experiment are graphically depicted in Figs. 5 and 6. The curves of the cumulative growth and sunlight measurements are shown in Fig. 5. Curves showing the weekly increase in height and the weekly sunlight intensity are pictured in Fig. 6. Sunlight radiation is expressed as m.e. of oxalic acid decomposed.

From the graphs of the cumulative sunlight record (Fig. 5), it can be seen that the one-half shade series received only 40 per cent of the full sunlight, while the three-fourths

Fig. 5. Cumulative growth and sunlight curves.



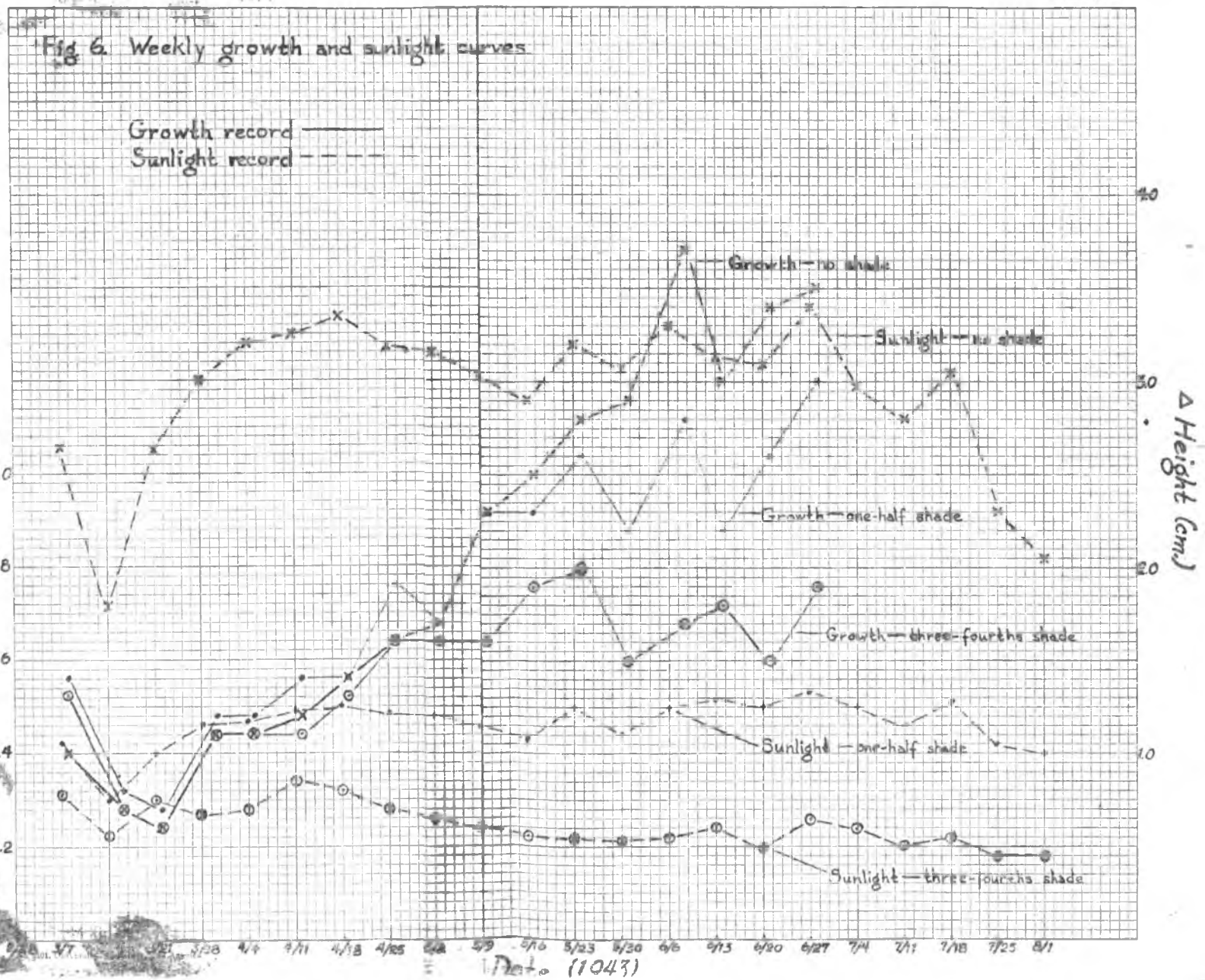


Fig 6. Weekly growth and sunlight curves

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shade series received about 20 per cent.

During the first half of the experiment the growth of the plants under the three light conditions appeared to be similar, but toward the end of the experiment the growth of the plants without shade appeared better than that of the shaded series (Fig. 5). In order to find out whether the differences in growth among the three series were significant or not, the significance of the differences was determined by the method of analysis of variance. In so doing, the growth period was divided into two parts--March 1 to May 3, and May 3 to June 28--and the increases in growth during these two periods were calculated. Table I contains the data showing the average increase in height during the two periods.

TABLE I

AVERAGE INCREASE IN HEIGHT OF COFFEE PLANTS
(in cm)

Series	Treatment	5/3-3/1	6/28-5/3
X	No shade	10.4	24.1
Y	One-half shade	11.6	20.0
Z	Three-fourths shade	10.6	13.9

TABLE II

STATISTICAL EVALUATION OF GROWTH DATA IN TABLE I

Period	X-Y(or Z)	Y-Z
5/3-3/1		
Difference to be significant at 5% level	1.36 cm	1.66 cm.
6/28-5/3		
Difference to be significant at 1% level	3.43 cm	3.91 cm.

Statistical evaluation of the growth data showed that during the early stages of growth, the coffee plants grew as well without shade as with shade (Table II). But, during the later half of the experiment, the growth of the plants without shade was significantly better than the growth of the plants under one-half and three-fourths shades. The growth of the plants under one-half shade was also significantly better than the growth of the plants under three-fourths shade. Even after making allowances for the amount of sunlight cut off by the glass panes (about 10 per cent), these results seem to be somewhat contrary to those reported by Arrillaga and Gomez (7) in Puerto Rico. These workers found that the growth of coffee trees in the field decreased as solar radiation increased and that the best growth was made under three-fourths shade.

The cause of the discrepencies between the results obtained in this experiment and those reported by Arrillaga and Gomez probably lies in the different nutritional requirements of coffee trees grown with and without shade, and not to the differences in sunlight intensity existing between that in Hawaii and that in Puerto Rico. Arrillaga and Gomez reported that during their investigation the total annual radiation ranged from 135,000 to 158,000 gram calories per square centimeter. At the Hawaiian Sugar Planters' Experiment Station, near the University of Hawaii, the sunlight radiation averages about 180,000 gram calories per square centimeter annually (10). These figures lead one to the

belief that whatever difference in sunlight intensity that exists between the two localities, it is not large enough to account for the different results obtained in the two localities. One might object that the plants used in this experiment were too young; but, it is the general observation that young coffee plants are more susceptible to intense sunlight than older plants. It is possible, however, that the duration of this experiment is too short. Yet, as indicated by the diverging tendency of the growth curves, it hardly seems likely that the shaded plants will ever catch up with the unshaded plants.

Returning to the question of nutrition, there were several indications that unshaded plants require a higher level of fertilization than shaded plants. Whenever nitrogen or potassium was omitted from the culture solution, the unshaded plants were always the first to show the effects of the omission. The lower percentages of nitrogen and potassium in the leaves of the unshaded plants (Tables IV and VII)--results which are believed to be due to greater utilization--lend further support to this view. Interestingly enough, in the experiment of Arrillaga and Gomez (7), although the plants under full sunlight accumulated greater amounts of nitrogen and potassium than under any other exposure, they appeared chlorotic. This seems to point to a condition of physiological upset in the unshaded plants. Indeed, Reed (61) has pointed out that synthesis of large amounts of carbohydrates has a growth-limiting and chlorophyll-deficiency effect in

leaves unless a supply of available nitrogen is maintained during the synthesizing process. All the preceding statements bring one to the opinion that it is likely that poor nutrition was a factor which prevented the unshaded coffee trees in Puerto Rico from making better growth. Burkholder (11) has stated: "For optimum growth of any green plant under a given set of conditions with respect to temperature, mineral nutrition, water supply, etc., there is a certain light exposure of optimum intensity, quality, and duration."

The curves in Fig. 6, showing the weekly increase in height and the weekly sunlight intensity, seem to offer additional evidence that the unshaded plants did grow better than the shaded plants during the last half of the experiment. As can be seen, during the later half of the experiment not only were the weekly growth increases larger for unshaded plants, but there seemed to be a tendency for the growth curves to parallel the sunlight curves. Because of the short period of observation, however, it cannot be concluded that the growth curve follows the pattern made by the sunlight curve. It is regrettable that more data along this line were not obtained.

Chemical Analysis of Coffee Leaves

The results of the chemical analysis of the coffee leaves are recorded in Tables III-VII. Moisture determinations are listed in Table III, nitrogenous fractions in Table IV, ratios of nitrogenous fractions to total or soluble nitrogen in Table V, carbohydrates in Table VI, and ash constituents in Table VII.

Dry matter content. From the figures in Table III, it can be seen that the dry matter content of the leaves is decreased by shading (38). The same trend is observable in all the light series. Shirley (64) has reported that the percentage of dry matter in tops of eleven species of plants increases with increasing light intensity.

The absence of potassium results in an increase in dry matter content, probably due to the necrotic tissues. Hartt (29) has reported that sugar cane plants grown without potassium have lower moisture contents than the control plants. On the other hand, phosphorus deficiency tends to lower the dry matter content.

TABLE III

MOISTURE CONTENTS OF COFFEE LEAVES
(on wet basis)

Series		Treatment	Moisture %	Dry Matter %
I	A	Complete nutrient, no shade	72.83	27.17
	B	" " , one-half shade	74.35	25.65
	C	" " , three-fourths shade	75.35	24.65
II	A	3 wks. before sampling, -N, no shade	73.39	26.61
	B	" " " " ", ½ shade	74.50	25.50
	C	" " " " ", ¾ "	75.00	25.00
III	A	3 wks. -N, followed by 3 wks. +N; no shade	72.58	27.42
	B	" " ", " " " " "; ½ shade	73.30	26.70
	C	" " ", " " " " "; ¾ "	74.10	25.90
IV	A	6 wks. before sampling -K; no shade	71.00	29.00
	B	" " " " "; ½ shade	72.84	27.16
	C	" " " " "; ¾ "	74.00	26.00
V		6 wks. before sampling -P; no shade	72.57	27.43
VI		6 wks. before sampling +2N; no shade	71.42	28.58
VII*		Complete nutrient, no shade	67.51	32.49
VIII*		-K, no shade	65.23	34.77
IX*		-P, " "	68.98	31.02

*Plants used in preliminary experiment. They were given respective treatments until definite deficiency symptoms appeared.

TABLE IV

NITROGEN FRACTIONS IN COFFEE LEAVES
(on dry basis)

Series	Total N %	Sol. N %	Ins. N %	NH_3 N p.p.m.	Amide N p.p.m.	NO_3 N %	Basic N %	a-NH_2 N %	Peptide N %
I A	2.72	.772	1.95	trace	2.0	.027	.514	.08	.06
B	3.18	.895	2.28	.1	8.8	.123	.520	.10	.16
C	3.44	.971	2.47	2.0	trace	.230	.494	.10	.12
II A	2.51	.705	1.80	.5	5.0	.019	.499	.06	.04
B	2.56	.679	1.88	.6	2.2	.024	.480	.07	.11
C	2.66	.680	1.98	.7	6.6	.057	.472	.06	.12
III A	2.44	.663	1.78	1.2	trace	.015	.464	.09	.02
B	2.59	.621	1.97	.8	1.2	.045	.444	.06	.03
C	3.04	.777	2.26	4.4	3.2	.105	.445	.11	.07
IV A	2.78	.821	1.96	.6	1.8	.021	.502	.07	.02
B	3.02	.804	2.22	1.2	12.6	.053	.495	.10	.08
C	3.32	.949	2.37	4.0	2.0	.078	.476	---	---
V	2.98	.830	2.15	1.5	trace	.063	.525	.10	.13
VI	3.47	1.193	2.28	6.6	3.8	.143	.623	.08	.19
VII	2.69	.715	1.98	.4	1.6	.031	.483	.08	.02
VIII	2.72	.878	1.84	trace	2.4	.035	.568	.08	.07
IX	3.77	1.921	1.85	7.8	6.4	.214	.773	.20	.28

Total nitrogen. The trend of the figures in Table IV indicates that total nitrogen increases with shading. Withholding nitrogen from the plants results in a decrease in the total nitrogen content and diminishes the differences in nitrogen content existing among the plants under different light treatments (series II). This result shows that a good proportion of the nitrogen in the shaded plants is storage nitrogen. As expected, increasing the external nitrogen supply increases the total nitrogen content of the coffee leaves.

Severe phosphorus deficiency (series IX) results in an abnormal accumulation of nitrogen. The figures for total and soluble nitrogen and the ratio of soluble to total nitrogen (Table V) show that this increase is due to an abnormal increase in soluble nitrogen. According to MacGillivray (41), phosphorus starvation causes tomato plants to accumulate nitrogen, chiefly in water-soluble forms. Clements et al. (15) found that phosphorus deficiency results in accumulation of total nitrogen in certain tissues of the sugar cane plant.

Potassium deficiency does not seem to affect the total nitrogen content of coffee leaves.

Soluble nitrogen. Table IV shows that with an ample supply of nitrogen soluble nitrogen increases with shading. The increase is due largely to the accumulation of nitrates. When nitrogen is withheld from the plants, there is a decrease in soluble nitrogen. Comparing the soluble nitrogen content of the unshaded leaves with that of the shaded ones of series

II, the larger value for the former is probably due to hydrolysis of insoluble nitrogenous compounds.

Lack of potassium increases the soluble nitrogen content of the leaves to some extent, the increase being due to an increase in the basic nitrogen fraction.

A severe lack of phosphorus results in an abnormal increase in soluble nitrogen, chiefly due to increases in the amounts of nitrate and peptide nitrogen. Basic nitrogen and alpha-amino nitrogen are also accumulated by the phosphorus-starved plants.

It is interesting to note that according to Table V, the ratio of soluble nitrogen to total nitrogen is about the same for all plants in series I regardless of light treatment. Other plants that did not show the effect of their treatments had about the same ratio. It is probable that this is an equilibrium phenomenon. If such be the case, any external stress that affects the nitrogen metabolism of the plant should alter the ratio; and, moreover, if the stress is not too severe and if the plant can remedy the effect, the plant will modify its metabolism so as to approach the previous value. Thus, when nitrogen is withheld from the plants, soluble nitrogen in the leaves is rapidly used up, thereby lowering the ratio of soluble nitrogen to total nitrogen (see series II C). With further lack of nitrogen, the insoluble nitrogenous reserves are hydrolyzed, resulting in an increase in the ratio (series II B). When all the insoluble reserves are depleted and transformed to soluble compounds, the ratio will tend to be close to the equilibrium figure

TABLE V

RATIOS OF NITROGEN FRACTIONS TO TOTAL NITROGEN OR TO SOLUBLE NITROGEN

Series	Sol. N/ total N	Ins. N/ total N	NO_3 N/ sol. N	Basic N/ sol. N	a-NH_2 N/ sol. N	peptide N/ sol. N
I A	.284	.716	.035	.666	.109	.082
B	.281	.719	.137	.581	.116	.175
C	.282	.718	.237	.509	.104	.125
II A	.281	.719	.027	.708	.089	.060
B	.265	.735	.035	.707	.097	.160
C	.256	.744	.084	.694	.091	.179
III A	.272	.728	.023	.700	.140	.030
B	.240	.760	.072	.715	.097	.042
C	.256	.744	.135	.573	.138	.094
IV A	.295	.705	.026	.611	.090	.026
B	.266	.734	.066	.616	.129	.104
C	.286	.714	.082	.502	----	----
V	.279	.721	.076	.633	.116	.154
VI	.344	.656	.120	.522	.068	.162
VII	.266	.734	.043	.676	.111	.028
VIII	.323	.677	.040	.647	.090	.080
IX	.510	.490	.111	.402	.104	.148

(series II A). If nitrogen deficiency becomes so severe that the protoplasm is injured, it is very likely that the ratio will be drastically changed. This has been found to be the case under severe potassium and phosphorus deficiencies (series VIII and IX).

Insoluble nitrogen. It is very interesting to observe that insoluble nitrogen in coffee leaves increases with shading and decreases when the external supply is cut off (Table IV). Hopkins (34) and Kraybill (38) have reported similar results for other plants. The responses of the plants in series II and III indicate that a substantial proportion of the insoluble nitrogen in shaded plants is in the form of reserve proteins. From the values for the nitrogen series and for the potassium and phosphorus deficient plants, it appears that protoplasmic nitrogen in coffee leaves makes up about 1.8 per cent of the dry weight.

Nitrate nitrogen. It can be seen from Table IV that the amount of nitrate nitrogen in coffee leaves shows a wide variation, ranging from a few thousandths of one per cent to over two-tenths of one per cent. Although sunlight is known to play an indirect role in the assimilation of nitrate by green plants (46), the low percentages of nitrate nitrogen in the leaves of plants grown without shade point to the importance of sunlight in the assimilation of nitrate nitrogen by coffee leaves. It is apparent that nitrate nitrogen increases with increase in shading and with increase in the external supply. When there is no nitrate in the culture

solution, the nitrate stored in the leaves is quickly used up by the plant (series II). On the other hand, when it is again supplied to the plant, the excess is stored again by the plant (series III). These results indicate that when there is an excess of nitrate the accumulation of nitrate by coffee leaves under adequate nutritional conditions is a normal phenomenon; and, the degree of accumulation is largely determined by the amount of solar energy available to the plants.

Phosphorus starvation results in an abnormal accumulation of nitrates. Other workers (21, 23, 62) have reported similar results with other plants. Eckerson (23) attributes the accumulation of nitrate to the role phosphorus plays in reducase synthesis.

According to Nightingale (48) nitrate is not absorbed freely by pineapple plants under field conditions when the supply of potassium is low. The low nitrate contents of the leaves in series IV seem to confirm Nightingale's observation. Hartt (30) has reported that reduction of nitrates by sugar cane plants does not seem to be affected by the absence of potassium.

Ammonia and amide nitrogen. From Table IV, it appears that ammonia nitrogen and amide nitrogen are not accumulated to any large extent by coffee leaves under the conditions of this experiment. The trend of the figures indicates that both types of nitrogen are found in larger amounts in shaded leaves. Both forms seem to accumulate when the nitrogen

supply is greater than the utilization. The lack of phosphorus results in increases in ammonia nitrogen and amide nitrogen. Richards and Templeman (62) have reported that phosphorus deficiency results in an increase in amide content in barley leaves.

In connection with the low ammonia nitrogen and amide nitrogen contents of coffee leaves, it may be of interest to mention the study of Clark (13) on the effect of ammonia and nitrate nitrogen on the composition of tomato plants. He found that more glutamine and asparagine are synthesized by ammonia-fertilized plants than by nitrate-fertilized plants. Amino nitrogen, other than glutamine and asparagine nitrogen, forms only a small part of the total amino nitrogen in ammonia-fertilized plants, but it makes up a relatively large part in nitrate-fertilized plants. It is possible that the coffee plant may behave in an analogous manner.

Basic nitrogen. The high percentage of basic nitrogen (Table IV) in the leaves of coffee plants indicates that this nitrogenous fraction can not be overlooked in a consideration of the soluble nitrogenous constituents of coffee leaves. Under normal conditions it often comprises more than half of the total soluble nitrogen fraction. It seems to show a slight decrease in amount with shading, but whether the decrease is significant or not is not known. A diminution in the external nitrogen supply also results in its decrease. An abnormal increase in its absolute amount results from the lack of phosphorus. Severe potassium deficiency seems to

cause some accumulation of basic nitrogen at the expense of insoluble nitrogen. A consideration of the amounts of basic nitrogen in the leaves and of its ratio to soluble nitrogen (Table V) indicates that of all the soluble nitrogen fractions determined in this study this fraction is probably the most stable. What part the basic nitrogen fraction plays in the metabolism of coffee leaves is not known. Its largest constituent, however, is likely to be caffeine, a xanthine derivative. Weevers (76) has expressed the belief that xanthine derivatives have their origin in the decomposition of proteins, and when the need arises are again used for synthesis of new proteins.

Alpha-amino nitrogen. Looking again at Table IV, it can be seen that as with most of the other soluble nitrogenous fractions in coffee leaves, alpha-amino nitrogen tends to increase with shading. Absence of an external supply of nitrogen results in a decrease in the alpha-amino nitrogen content. The lack of potassium does not seem to affect the concentration of alpha-amino nitrogen, but the absence of phosphorus results in a large increase in its amount.

Peptide nitrogen. A trend similar to that of the alpha-amino nitrogen is shown by the peptide nitrogen fraction. It increases with shading, and decreases when the external supply of nitrogen is cut off. With it also, the lack of potassium does not seem to affect its amount within the coffee leaves, while the absence of phosphorus results in an abnormal accumulation of peptide nitrogen. Its relative amount

(Table V) seems to show a greater fluctuation to imposed conditions than that of alpha-amino nitrogen.

Soluble sugars. Turning now to Table VI, one can see

TABLE VI

CARBOHYDRATE FRACTIONS IN COFFEE LEAVES
(on dry basis as dextrose)

Series	Soluble Sugars %	Starch %	Hemicellulose %
I A	6.23	3.71	7.38
B	5.83	.32	7.65
C	5.05	.16	8.30
II A	6.65	4.60	6.98
B	6.42	2.88	8.35
C	5.48	1.28	8.80
III A	5.60	2.18	7.65
B	5.95	1.46	7.80
C	5.42	.10	8.48
IV A	8.55	4.02	7.35
B	8.02	.16	8.20
C	6.10	.16	8.00
V	6.42	1.24	6.90
VI	5.22	.16	6.88
VII	5.48	.34	6.88
VIII	5.52	.16	7.00
IX	4.25	.16	6.98

that the soluble sugar content shows a decided decrease with increase in shading. Kraybill (38) and Hopkins (34) have reported similar results with other plants. Omitting nitrates from the culture solution causes increases in the soluble sugar content in all light treatments (18). A probable reason why the leaves of series III A contain less soluble sugars than that of series III B is that when nitrate was again supplied to the plants of series III A it was vigorously reduced at the expense of sugar and starch fractions. The starch content of series III A and the low sugar content

of series VI seem to support this explanation. There is some accumulation of soluble sugars when nitrogen is withheld; but, when potassium is omitted from the culture solution (series IV), the result is a very marked increase in the soluble sugar content. Hartt (30) has reported that a partial deficiency of potassium causes an accumulation of reducing sugars and a decrease in sucrose in sugar cane. It should be noted that under conditions of severe deficiency of potassium (series VIII) soluble sugars do not accumulate in the leaves (30). Nightingale (47) has pointed out that at the outset of potassium deficiency, carbohydrates accumulate; and, with further lack of potassium, carbohydrates decrease. In series V, the lack of phosphorus does not seem to affect the soluble sugar content. It is generally known that phosphorus starvation results in an accumulation of soluble sugars (21, 37, 41). It is possible that the coffee plants in series V had enough phosphorus to carry them through the period they were not supplied with phosphorus. Under conditions of severe phosphorus deficiency, however, the leaves show a substantial reduction in soluble sugars (series IX).

Starch. A very marked decrease in starch content with increase in shading is shown by coffee leaves (Table VI). Like the soluble sugars, cutting off the nitrogen supply causes an accumulation of starch within the leaves. Restoration of the supply seems to result in the utilization of the starch that had been accumulated (series III). At first

the lack of potassium seems to cause a small increase in the starch content (series VIII) (47). Under phosphorus deficiency, the starch content of the leaves shows a tendency toward a reduction (21, 37).

Hemicellulose. Unlike the other carbohydrate fractions, the hemicellulose fraction shows a decided tendency toward an increase with increasing shade (Table VI). There seems to be no explanation why the coffee plant behaves in this manner. Kraybill (38) found that shading causes a decrease in hydrolyzable material in apple and peach trees.

Potassium. The results of the potassium analyses are recorded in Table VII. From the table, it can be seen that the potassium content of the leaves increases with increase in shading. All light treatments show this trend. This condition is believed to be the result of luxury consumption. Omitting potassium from the culture solution results in a reduction in its concentration in the leaves. Incidentally, the plants in series VI received their increase in nitrogen as KNO_3 and $Ca(NO_3)_2$. That is why the potassium content of series VI is greater than that of series I.

As has been mentioned before, the leaves of the plants in series IV A, containing 1.62 per cent potassium (dry weight basis), were showing symptoms of potassium deficiency at the end of the experiment; while those of series IV B with 1.82 per cent potassium did not show such symptoms. The value of 0.76 per cent for series VIII and the 4.06 per cent value for series I C show how wide a range in potassium

content coffee leaves can have.

Calcium. There seems to be a tendency for the calcium content of coffee leaves to increase with shading. Absence of

TABLE VII

ASH CONSTITUENTS IN COFFEE LEAVES
(on dry basis)

Series		% K	% Ca	% P
I	A	2.89	1.46	.20
	B	3.40	1.60	.18
	C	4.06	1.66	.18
II	A	2.84	1.40	.20
	B	3.10	1.53	.20
	C	3.52	1.58	.19
III	A	3.06	1.40	.20
	B	3.58	1.56	.20
	C	4.06	1.58	.19
IV	A	1.62	1.50	.18
	B	1.82	1.58	.18
	C	2.49	1.80	.21
V		3.20	1.47	.10
VI		3.46	1.72	.20
VII		2.74	1.33	.15
VIII		.76	1.52	.16
IX		2.84	1.23	.06

potassium in the culture medium seems to result in an increase in calcium content. It is not known whether this is due to increased calcium absorption or to an increase in dead tissues. Hartt (29) and Clements et al. (15) have reported that sugar cane plants grown without potassium accumulated more calcium than those with potassium.

Phosphorus. Unlike potassium and calcium, the phosphorus content of the leaves tends to remain fairly constant irrespective of light treatment. Omitting phosphorus from the nutrient supply causes a marked decrease in the phosphorus

content. As mentioned previously, the leaves of the plants in series IX with 0.06 per cent phosphorus (dry weight basis) showed symptoms of phosphorus deficiency at the time they were picked. Those of series V with 0.10 per cent phosphorus did not show any visible effect of lack of phosphorus.

The behavior of the phosphorus deficient plants, as revealed by the results of the chemical analysis for the various nitrogenous fractions, shows that lack of phosphorus causes a very serious breakdown in the normal process in nitrogen metabolism. The fact that insoluble nitrogen content of the minus-phosphorus plants shows hardly any change indicates that the breakdown lies in the anabolic process of nitrogen metabolism.

SUMMARY

Coffee plants (Coffea arabica L.) were grown in water culture under three different sunlight intensities.

It is concluded that under suitable nutritional conditions, the accumulation of nitrates in the leaves of the coffee plant is a normal process, and that the amount stored at a certain period is determined largely by the amount of solar energy available to the plant at that time. If the supply of nitrate nitrogen is larger than its demand, the excess is stored by the plant.

The results obtained show that under the conditions of this experiment, with adequate nutritional conditions coffee plants grew better without shade than with heavy shade.

Plants grown without shade and under one-half shade appeared much hardier than those grown under three-fourths shade. The number of leaves per plant decreased with increase in shading, while the leaf size increased with shading. Unshaded plants had larger trunks and larger root systems than the shaded plants.

There were indications that unshaded plants have a larger nutrient requirement than shaded plants.

Chemical analysis of the leaves showed that increases in shading resulted in increases in total nitrogen, soluble nitrogen, insoluble nitrogen, ammonia nitrogen, amide nitrogen, nitrate nitrogen, alpha-amino nitrogen, peptide nitrogen, and hemicellulose; and resulted in decreases in dry matter, soluble sugars, and starch. Potassium, and to a lesser

extent calcium, tended to increase with shading. Phosphorus content did not seem to be affected by shading.

When nitrate was withheld, sugars and starch accumulated in the leaves.

Severe potassium deficiency resulted in an increase in the soluble nitrogen content. The increase was due principally to an increase in the basic nitrogen fraction. At the outset of potassium deficiency, soluble sugars accumulated but later fell back to normal values.

Severe phosphorus deficiency resulted in abnormal increases in total nitrogen, ammonia nitrogen, amide nitrogen, nitrate nitrogen, basic nitrogen, alpha-amino nitrogen, and peptide nitrogen. These results point to a serious breakdown in the nitrogen metabolism of phosphorus-starved plants. The breakdown seems to be located in the anabolic process. Among the soluble nitrogenous fractions, nitrate nitrogen showed the greatest increase. Soluble sugars and starch were decreased by the lack of phosphorus. Hemicellulose was not affected.

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