Summary

1. 6-Aldehydo-coumarin or *p*-coumaric aldehyde, melting at 189°, has been prepared by the application of Reimer and Tiemann's reaction on coumarin; it gives a hydrazone, an oxime and a semicarbazone, responds to Claisen's reaction and undergoes benzoin condensation in the usual manner.

2. Interesting azo-methine dyes have been obtained by the condensation of 6-aldehydo-coumarin with (1) mono-amines such as *p*-toluidine, β-naphthylamine, *p*-nitraniline and amino-azobenzene; (2) diamines such as benzidine, *o*-, *m*- and *p*-phenylenediamines; (3) dyes such as rosaniline, safranine and chrysoidine which contain free NH_2 groups.

3. Beautiful triphenylmethane dyes have been obtained by condensing the aldehyde with dimethylaniline and *o*-cresotinic acid.

4. Pyronine dyes have been obtained by condensing the aldehyde with diethyl-*m*-aminophenol and also with such hydroxy compounds as resorcinol, hydroquinone and gallic acid in the presence of sulfuric acid. The C=O group in the lactone ring as well as the aldehyde group reacts in these condensations except in the case of diethyl-*m*-aminophenol.

CALCUTTA, INDIA

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

EFFECTS OF LIGHT UPON NITRATE ASSIMILATION IN WHEAT[1]

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In a previous article[1a] on temperature effects in the metabolism of wheat the senior writer stressed the need of determining the adequacy of spectral balance when the illumination is wholly from an artificial source. The present paper deals with the assimilation of nitrate by young wheat plants, as affected by differences in spectral distribution and total intensity of light. As grown in our climatic chambers under water cells, the Marquis variety of spring wheat, which we have employed solely, requires between 1200 and 2000 foot candles of illumination at about 18° in a twelve-hour day, from Mazda C electric lamps. This estimate is based upon the maximum yield of dry matter. It is not clear, however, that this is the most significant index of optimal illumination, as will appear in a test to be presented presently. In this case more nitrate was assimilated, but at the expense of tissue production, when the Mazda lamps were supplemented by the visible and long ultraviolet rays from a carbon arc.

[1] Published with permission of the Director of the Wisconsin Agricultural Experiment Station.

[1a] W. E. Tottingham, *Plant Physiol.*, **1**, 307 (1926).

While it is well known that short ultraviolet light is generally injurious to plants, little is known concerning specific effects of blue-violet and long ultraviolet rays. Plant physiologists have long known that the tropic effects of light are due primarily to the shorter wave lengths of the visible spectrum, but there is little, if any, published work showing the metabolic response of the organism to this particular factor.

Experimental Part

Our preliminary experience dealt with selective effects of greenhouse glass upon irradiation of maize (*Zea mais*) by sunlight. Seedlings of the Golden Glow variety from ordinary field stock were reared in sand in a greenhouse without permitting direct access of the sun's rays. Uniform selection as to appearance and size was then made for transference to water cultures of one plant to each jar of about 0.9 liter (1.0 quart) capacity. The nutrient solution was one designed to supply a high proportion of nitrate, containing 0.2% of sodium nitrate in 0.3% of total salts. A modification of this formula contained 0.2 as much nitrate. At intervals of three to four days the residual solutions were replaced by fresh portions. On sufficiently clear days a chosen section of the cultures was placed outside the greenhouse for direct exposure to solar radiation during about one and five-tenths hours at midday, taking care that the temperature change was gradual and similar for all cultures. Each section included eight plants. A second test was made in cooler weather, when the difference in irradiation was possible only on warmer days. The temperature was 6.0 to 12.0° warmer in the house than out of doors during periods of exposure in both tests, and the vapor pressure deficit of the atmosphere was 60 to 70% greater in the former location in the second test. In so short a period of exposure temperature would hardly be a critical factor in metabolism and, furthermore, maize appears to be relatively stable in composition with varying environment.[2] Moreover, general experience has shown a conservation of carbohydrates at lower temperatures, with decreased percentage of nitrogen. It might be expected that increased transpiration would favor the absorption of nitrate in the cultures continuously housed, but the work of Muenscher[3] indicates that these functions are independent of each other. There are thus small grounds for anticipating increase of nitrate assimilation in the exposed plants.

At the expiration of each culture period the fresh tissues were extracted with water and analyzed by methods which are specified elsewhere.[4]

[2] W. E. Tottingham, *Science*, **59**, 69 (1924); W. E. Tottingham and H. W. Kerr, *Plant Physiol.*, **1**, 415 (1926).

[3] Muenscher, *Am. J. Botany*, **9**, 311 (1922).

[4] Committee on Methods of Chemical Analysis, Recommendations, *Plant Physiol.*, **1**, 397 (1926); **2**, 91, 195, 205 (1927).

The residual nutrient solutions were restored to their original volumes for the determination of nitrate, Strowd's modification of DeVarda's method[5] being employed here for both extracts and nutrient solutions. In view of the large variability in physiological behavior of maize, it was deemed inexpedient to deal with smaller units than the total mixed tissue and solution of each section of cultures. Hence, one can hardly express variability of the results. It should be noted that the radiation in the second test was only 40% of that in the first and the average daily maximum temperature out of doors was 13.9° lower in the former case. The results with maize are assembled in Table I.

TABLE I

RELATION BETWEEN SOLAR ILLUMINATION AND NITRATE ASSIMILATION IN MAIZE AS AFFECTED BY GREENHOUSE GLASS

Exposure to direct sunshine	Radiation outside green-house, cal.	Nitrate supply, mg. per liter	Nitrate absorption, %	Dry matter, %	Composition of dry matter: Glucose, %	Sucrose, %	Protein N Insol., %	Protein N Sol., %	Basic N, %	Nitrate N, %
Aug. 10–25	1237[a]	63	86	9.8	1.8	16.4	2.1	0.8	0.08	0.9
None	1237	63	79	10.0	2.6	16.6	2.0	.6	.06	.8
Aug. 10–25	1237	299	18	9.3	1.5	15.8	1.8	.9	.09	.6
None	1237	299	14	11.6	4.9	9.4	2.0	.6	.05	.3
Oct. 5–17	506	207	2	6.3	...	5.8[c]	2.6	.9	.05	.9
None	506	207	−8[b]	8.9	...	2.3[c]	2.5	.6	.11	.8

[a] Calories for two and three days' exposure after last change of nutrient solution, 221 and 194, respectively.

[b] Excretion from the plant.

[c] Total sugars after hydrolysis.

Other tests were made with spring wheat (*Triticum sativum*) of the Marquis variety, using the same form of nutrient solution as before. A preliminary trial shows the range of variability which may be expected in results of this character. The seed used was a pure line selection provided by Dr. J. G. Dickson of the Department of Plant Pathology in this institution. Seedlings four days old were irradiated for sixteen hours per day by tungsten incandescent lamps at an intensity of 200 foot-candles, with the use of a blower to reduce heat effects. The temperature at the surface of the culture jars ranged from 17 to 18°. After twenty-one days' exposure, the entire plant was dried at 70° for analysis. These results are given in Table II. In all of the tests on wheat it has been impracticable to report variability, because of the limited repetition of culture treatment. With water cultures generally, forty plants were supported in a 3.5-liter jar and with soil cultures reared to maturity, ten or twelve plants per pot of 3.5 kg. of soil. Even with duplicate culture treatment the yield was insufficient for more than single analyses of immature plants.

[5] Strowd, *Soil Science*, **10**, 333 (1920).

TABLE II

VARIABILITY OF YIELD AND COMPOSITION IN WATER CULTURES OF WHEAT ILLUMINATED AT 200 FOOT CANDLES BY TUNGSTEN LAMPS

Series	Dry matter, g.	Protein N, %	Protein, g.	Other sol. N, %	NO_3 absorption, mg. per liter	Total sugars %	Total sugars g.
A	11.40	1.95	1.39	1.33	52	1.72	0.20
B	11.35	1.88	1.32	1.29	58	1.53	.17

In test 2 upon wheat, a 500-watt Mazda lamp was supplemented by a carbon arc for increasing the proportions of shorter visible wave lengths and the long ultraviolet. The latter was a Thomsen inclosed lamp of 7.5 amp. capacity, operating on 110 volts A.C. Its globe was replaced by a conical tin reflector similar to those used with the tungsten lamps. Measurements with a Sharp-Millar photometer employing a selective screen for the red rays showed the intensity of illumination from the arc to be about 0.25 that from the filament lamp. Comparative measurements at various wave lengths were made by means of a spectrophotometer and the results are quoted hereafter.

This test covered a period of ten days with seedlings which had attained an age of one week. By daily use of the arc lamp for one to one and one-half hours the intensity of red rays was increased about 2.0%, corresponding to about 150% increase in the long ultraviolet. The interposing of common window glass between lamps and plants eliminated effectiveness of rays shorter than about 3000 Å. At the termination of irradiation the tissues of the plant tops only were well ground and extracted with water in the fresh state, determining nitrate (as hereafter) by the colorimetric method of Burrell and Phillips.[6] The data of this series are in Table III, with graphic presentation in Fig. 1.

TABLE III

INFLUENCE UPON THE METABOLISM OF WHEAT OF INCREASING THE SHORTER WAVE LENGTHS OF LIGHT ABOVE 3000 Å. ILLUMINATION OF 200 FOOT CANDLES. INCREASE OF ULTRAVIOLET BY THE CARBON ARC, 200%

Source of illum.	Yield of dry matter, g.	Glucose, %	Sucrose, %	Total sugar, g.	Insol. prot. N, %	Sol. prot. N, %	Total prot., g.	Basic N, %	Amino N, %	Nitrate N, %	NO_3 abs., mg. per liter
Mazda C	13.0	1.4	2.7	0.53	1.9	0.6	2.04	0.2	0.2	1.0	6
Mazda + arc	10.3	1.3	1.8	0.32	1.9	.7	1.75	.2	.5	0.8	16

The radiation curves in our figures were derived from the following sources: spectral energy distribution of the 1000-watt gas filled tungsten lamp, from data supplied through correspondence by Dr. W. E. Forsythe of the Nela Research Laboratories, whose data were derived from color temperatures; the graphs of Coblentz, Dorcas and Hughes[7] for the

[6] Burrell and Phillips, *J. Biol. Chem.*, **65**, 229 (1925).

[7] Coblentz, Dorcas and Hughes, *Sci. Paper Bur. Stds. No. 539*, **1926**.

emission spectrum of the carbon arc, as determined by a spectrometer and thermopile. Our own measurements of relative intensity of visible radiations from the tungsten lamp and carbon arc between 4060 and 6720 Å. were made by means of a Konig-Martens spectrophotometer. This instrument was made available through the kindness of Professor J. R. Roebuck of the Department of Physics, this institution. As a base of comparison we have employed our measurements of light intensity from the two sources by means of a Sharp-Millar photometer, with a screen of potassium dichromate, assuming that the observed difference was valid for wave length 6000 Å.

Relation of irradiation to assimilation of nitrate, wheat.

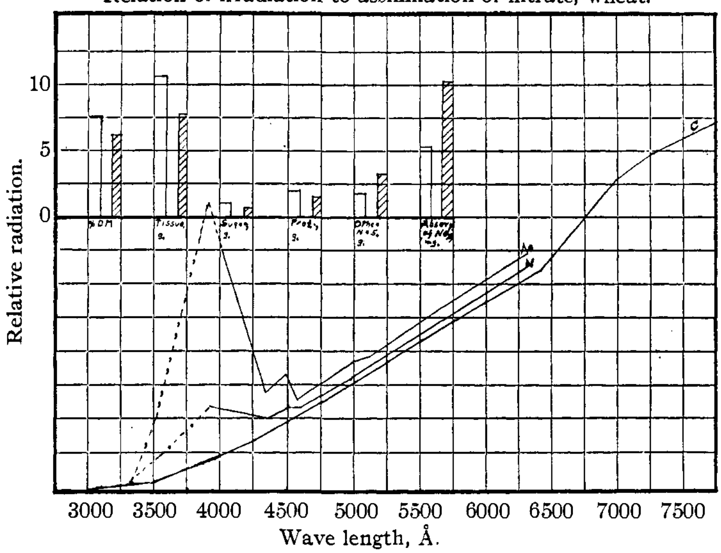

Fig. 1.—Constructed from the data of Table III. Line C shows the spectral distribution of radiation from the Mazda lamps. The radiation supplied by the additional use of the carbon arc is shown by line Ae. Curve Af shows the effective radiation based upon relative times of operation of the two types of lamps. In the altitudinal graphs clear areas represent responses to Mazda lamps, hatched areas those to the combined lamps.

The comparison in the long ultraviolet region is determined from Table 2 and Fig. 2 of Coblentz and associates. It is apparent from our Fig. 1 that the arc added about 3% to the intensity of visible radiations from the filament lamp and from 0 to about 300% in the long ultraviolet, with a maximum at about 3900 Å.

A further test was made of the effects of long ultraviolet radiation. In this case rays shorter than 3000 Å. were eliminated as before, while in a parallel group of cultures rays shorter than 4000 Å. were withheld by a special glass.[8] It was necessary, of course, to compensate for inequalities in light transmission by the different glasses, as given in the publication cited and by our own measurements with a Sharp-Millar photometer. The distances between the light filters and plants were adjusted accordingly. Water cultures reared in the greenhouse for seventeen days were irradiated for sixteen days. The intensity of 200 foot candles from the tungsten lamp was increased about 4.0% in the red rays by the arc and about 400% in the long ultraviolet. Without the use of special means to dispose of

Relation of irradiation to assimilation of nitrate, wheat.

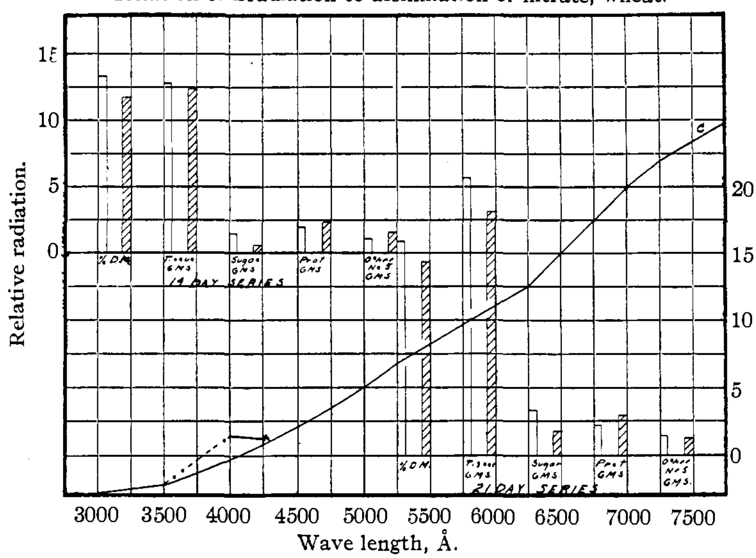

Fig. 2.—Constructed from the data of Table VI. Curve C shows the spectral distribution of radiation from the Mazda lamp. Curve A shows part of the additional radiation when the arc lamp was used. The latter increased the red rays about 0.5%, the long ultraviolet 50%. In the altitudinal graphs clear areas represent responses to the Mazda lamp, hatched areas those to the combined lamps.

heat it was not possible to irradiate at higher intensities, but the temperature was here maintained at 16.0 to 17.0°. The leaves receiving the more restricted radiation were wider than the others but turned yellow toward the close of exposure. Table IV shows the data obtained from analysis of the tops only.

It should be noted that for such tests as this, in which only a part of the plants is considered and the nutrient solution is renewed, one is not justified in expecting the plant analysis to conform to variations in the nutrient solution. In other words, the data do not permit the striking of balances between absorption from the solution and accumulation in

[8] Noviol, Bur. Standards, *Tech. Paper*, **119**, Fig. 24, **1919**.

TABLE IV

INFLUENCE OF LONG ULTRAVIOLET RAYS ON THE METABOLISM OF WHEAT AT 200 FOOT CANDLES, THESE RAYS FROM THE TUNGSTEN LAMP BEING INCREASED 400% BY THE CARBON ARC

Quality of light, Å.	Yield of dry matter, g.	Yield of dry matter, %	Glucose %	Sucrose, %	Total sugar, g.	Insol. prot. N, %	Sol. prot. N, %	Total prot. g.	Other org. N, %	Nitrate N, %	NO_3 absorption, mg. per liter
Above 4000	11.8	11.8	1.0	1.7	0.32	2.2	1.0	2.33	1.0	1.5	29
Above 3000	11.3	11.5	0.9	1.7	.30	2.3	1.1	2.36	0.9	1.3	52

the plant. A more complete check on this issue will be desirable in future work.

In a succeeding test the variability in illumination was made still greater, glass G34Y eliminating wave lengths shorter than 6000 Å. (orange), Noviol A limiting the effects chiefly to the visible portion of the spectrum, or to rays greater than 4000 Å., and Noviol O withholding rays shorter than 3700 Å. in the ultraviolet. The further use of Celo glass extended the radiation well into the ultraviolet, to about 2800 Å. Seedlings five days old in sand cultures were irradiated for nineteen days by lamps at about 300 foot candles' intensity and nine days further by sunlight in addition to a total estimated intensity of about 1200 foot candles. The plants deprived of yellow to violet light developed broader leaves but were more flaccid than the others. They were also etiolated and susceptible to attack by mildew. Under Celo glass the plants were erect, with narrow leaves, while in the other cases they appeared to be normal. The temperature here ranged from 17.0 to 20.0° and the tissues were analyzed from the fresh state. Data of this series appear in Table V.

TABLE V

INFLUENCE OF SPECTRAL PORTIONS UPON THE METABOLISM OF WHEAT AT AN AVERAGE INTENSITY OF ABOUT 300 FOOT CANDLES, TUNGSTEN AND ARC LAMPS SUPPLEMENTED BY SUNLIGHT

Quality of light, Å.	Yield of dry matter, g.	Yield of dry matter, %	Glucose, %	Sucrose, %	Total sugar, g.	Protein N, %	Protein g.	Other organic N, %	Nitrate N, %
Above 2800	8.2	13.5	2.2	3.9	0.50	2.4	1.23	2.2	0.2
Above 3700	11.4	11.9	2.7	2.6	.60	2.4	1.70	1.7	.3
Above 4000	11.1	11.8	1.9	3.6	.61	2.4	1.64	2.0	.3
Above 6000	9.5	12.1	3.0	4.4	.70	2.1	1.33	1.6	.8

It appeared that the planes of illumination thus far employed were probably deficient for full photosynthetic activity by the wheat plant, and that it would therefore be desirable to test the effect of spectral balance at much higher planes of light intensity. The adequacy of illumination was tested by the yields of dry matter in the climatic chambers already described,[1a] with the modification of covering the water cell by a sheet-iron dome adapted to forced ventilation and installing reflectored lamps therein. Differences in light intensity were secured by changing the elevation of culture jars on the rotating table. The cultures were irradiated for sixteen hours daily and the temperature was maintained at about 15.0°. With the further control of relative humidity at a uniform value of about 70.0%, the only markedly variable environmental factor was light intensity. Three tests with water cultures in periods of irradiation from 9.0 to 17.0 days showed, both by yields and sugar content, that 600 foot candles was much inferior to 1200 foot candles from this source of illumination. Other cultures on soil were continued for twenty-one and thirty-one days, at 13.0 to 14.0°, and with the light intensity ranging from about 2000 to 7000 foot candles in one case, and 2250 to 8000 foot candles in the other. In this case arc lamps were used at a constant distance through glass with an increase of about 0.4 to 0.1% in the red rays and about 40 to 50% in the long ultraviolet. The shorter test gave slight indication of deficiency of the lower plane of illumination but the longer one showed no difference either in yields of dry matter or percentage of nitrogen therein. These results indicate that the optimal range of this form of radiation for wheat, at the temperature here effective, lies between 1200 and 2000 foot candles and probably nearer to the latter value, as measured by yield of tissue.

On the basis of the preceding results a final test was made of the effect of supplementary radiation in the shorter wave lengths at total intensities ranging from about 1200 to 5000 foot candles as the plants increased in height. Sand cultures reared for twenty-one days in the greenhouse were subjected to controlled illumination in the chambers at about 18.0° for periods of fourteen and nineteen days. The increase of radiation due to the arc was about 0.5 to 0.8% in the red rays and 50 to 80% in the ultraviolet. In this case the tissues were dried at 98° before analysis. The data are given in Table VI, supplemented by Fig. 2.

Table VI

Influence upon the Composition of Wheat of Supplementing the Tungsten Lamp by the Carbon Arc above 3000 Å. Total Illumination 1200 to 5000 Foot Candles. Increase of Ultraviolet by the Arc, about 50%

Source of illum.	Dry matter, %	Dry matter, g.	Glucose, %	Sucrose, %	Total sugar, g.	Protein, g.	Protein N, %	Other org. N, %	Nitrate N, %
Tung. lamp, 14 days	13.1	12.9	8.2	0.3	1.10	1.75	2.2	1.2	0.3
Tung. + arc, 14 days	11.7	12.3	0.8	0.3	0.14	2.32	3.0	1.5	.4
Tung. lamp, 21 days	16.2	20.5	6.8	6.4	2.71	2.07	1.8	0.9	.3
Tung. + arc, 21 days	14.2	18.7	4.5	2.4	1.29	2.73	2.3	2.4	.2

Discussion

The data with maize show reduction of efficiency in the assimilation of nitrate and synthesis of soluble protein, and partly also of basic forms of nitrogen, by passage of sunlight through greenhouse glass. In the first test, at least, the sugar content did not vary sufficiently to account for the above difference. It seems probable that the results were due to deficiency of the shorter wave lengths of light under glass, for, as is well known, these are highly absorbed. Some authorities, such as Hovestadt,[9] give as the transparency of ordinary crown glass 10.0 cm. thick 90 to 95% for red to yellow, 70 to 80% for blue to violet and 60% or less for long ultraviolet. Stone[10] made tests on the diminution of chemical activity of light in passing through greenhouse glass. We understand that he followed the degree of oxidation in exposed solutions of ferric oxalate. The decrease was 14% in July and 43% in December.

From the test of variability with wheat it would seem that one could attach significance to variations of 10.0% and more in the composition of the plants and of twice this extent in the absorption of nitrate.

From the results of the second test with wheat it is apparent that the increase in proportion of long ultraviolet and visible blue-violet above that in the spectrum of the Mazda C lamp markedly increased the absorption of nitrate, and to a less extent enhanced the synthesis of amino forms of nitrogen. This activity is reflected in decreased content of nitrate and sugars in the plants receiving the more complete radiation. It will be noted that the differences in the absorption of nitrate are not accounted for by differences in the composition of plant tissue. This apparent discrepancy can well be explained by the fact that we have no data on nitrate absorption from the previous portions of nutrient solution, and the added fact that the root tissue was not analyzed. Computations based on the data of Coblentz, Dorcas and Hughes[7] and our own tests of spectral distribution indicate that the energy of wave lengths 3500 to 4500 Å., as related to those from 4500 to 7500 Å., or from the region of long ultraviolet to short blue waves to that from short blue to short infra-red, had a ratio of about 1:6 with our combined lamps as compared with 1:3 in sunlight. Thus a considerable increase in the proportion of shorter wave lengths of light produced a significant increase in protein synthesis.

From the third test (Table IV) it appears that the long ultraviolet rays as here effective were conducive to increased absorption of nitrate without promoting its assimilation. Final conclusions on this matter must await further trials. In the succeeding test the rays from 3700 to 4000 Å., or just below the range of the visible spectrum, were not found to be effective in this manner from the data on tissue. On the other hand, light extending into the middle ultraviolet considerably reduced the yields of tissue and of protein therein without sensibly affecting the composition.

[9] Hovestadt, "Jena Glass and its Scientific and Industrial Applications," transl. by J. D. and A. Everett, London, **1902**, pp. 47, 50.

[10] Stone, Mass. Agric. Expt. Station, *Bull. 144*, **1913**.

This effect should probably be ascribed to the well-known injurious action of shorter ultraviolet rays upon living matter. Absence of rays shorter than orange led to reduced yields of tissue and protein. The relative accumulation of nitrate and sugars in this case indicates inefficiency in protein synthesis.

While the foregoing tests might be criticized as inconclusive, due to the relatively low planes of total light intensity, this cannot apply to the final trials. The illumination here exceeded the intensity already reported as producing a maximum yield of plant tissue. Although the effect of the arc upon total intensity was here very small (about 0.5% increase in the red rays, and 50% in the ultraviolet), a considerable increase of protein at the expense of sugars is evident. Again, this process was accompanied by a decline in the production of tissue. It was deemed impracticable to follow the absorption of nitrate from sand cultures. Attention is called to the increased percentage of water in plant tissue subjected to increase of the shorter wave lengths of light, excepting the effect of shorter ultraviolet.

Summary

1. Data are presented from the growth of maize in water cultures during late summer. These indicate that the absorption of nitrate and synthesis of protein are promoted by light rays which are absorbed by greenhouse glass under the conditions here effective.

2. Data are presented from the growth of wheat in sand and water cultures which were irradiated by electric lamps for periods of ten to twenty-one days. With total intensities of 200 to 1200 foot candles and upward the long ultraviolet region was increased about 50 to 400% by use of carbon arcs. The concomitant increase in red-orange rays was about 0.5 to 4.0%, with unmeasured increases in the shorter wave lengths of the visible spectrum.

3. In the early growth phases of wheat, relatively limited increases in the proportion of shorter visible light rays greatly enhance the absorption of nitrate and significantly increase the synthesis of protein. These effects are accompanied by small increments of the percentage of water in the tissue and declines in the yields of sugars and total dry matter. Long ultraviolet rays seem to be effective in the absorption of nitrate, but the present results do not establish any further role.

4. The proportion of blue-violet rays in our artificial light was considerably smaller than in sunlight. Further study is desirable with sunlight or a similarly balanced spectrum.

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