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

Photochemical Responses of the Wheat Plant to Spectral Regions

BY E. J. LEASE AND W. E. TOTTINGHAM[1]

An earlier paper[2] reported increased absorption of nitrate by the young wheat plant when exposed to radiation from the carbon arc, as a supplement to winter sunlight. This response was later[3] obtained to the extent of 30%, with plants three weeks of age, by supplementing the mazda lamp with an arc of high amperage. Refinement of characterization for radiation of the later test gives an increase to 15-fold in radiation of the region 3100–4000 Å., by use of the arc, or to essentially 6% of the total between 3100 and 14,000 Å. In the region 4000 to 5000 Å. the increase was 270%, or to essentially 12% of the total. Apparently the value originally attributed[4] to this radiation was too high and should be revised to about 1200 f. c.

In the earlier study nitrate absorption was associated with the appearance of organic forms of nitrogen at the expense of sugar content and yield of dry matter. The present paper deals in greater detail with compositional responses of the plant to increases in proportion of the shorter rays of light.

Experimental

By the use, under water cells, of selective filters supplied by the Corning Glass Works, the spectrum emitted by the mazda lamp was altered to exclude radiation shorter than about either 3900 or 5200 Å. Space beneath was inclosed to exclude daylight. A range comparable with sunlight was obtained by use of the sunlight lamp[5] installed above a water cell of Corex D glass, and with a frosted pane of this glass to reduce the intensity of radiation. Adjustment of lamps was made to give, at the predominant leaf level, essentially equal intensities of about 1200 f. c. Forced ventilation of the plant chambers and surrounding greenhouse maintained a temperature range of essentially 17–18°. Earlier tests[6] placed these values of temperature and radiation as fairly favorable for the young wheat plant, with reference to production of dry matter. The plants were exposed nightly to a 15-hour period of irradiation, with a flow of about 1 cm. depth over the water cell.

A comparison of the forms of radiation transmitted to the plants is shown in Fig. 1, together with a distribution curve for mid-day, summer sunlight.[7] These graphs show marked deficiency

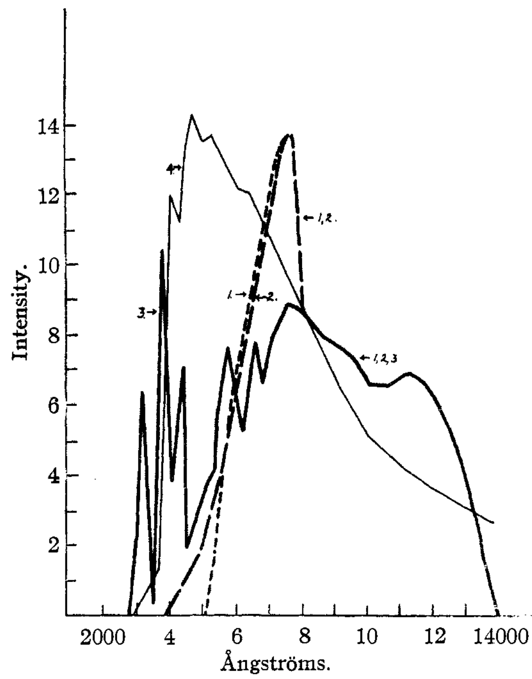

Fig. 1.—Effective radiation: 1 = No. 349 filter - - - - -; 2 = Noviol O filter —— ——; 3 = Sunlight lamp ━━━━; 4 = Unfiltered sunlight ——.

of blue-violet and discontinuity of ultraviolet in our substitute for sunlight as compared with the normal. As shown in Fig. 2,[8] the cutoff of filters under the sunlight lamp conformed closely to the lower limit of sunlight. The total effective radiation was measured by a thermopile supplied for the purpose by the Eppley Laboratory. Distribution of the radiation is given in Table I.

Irradiation was continued daily for three weeks

(1) With collaboration from E. R. Frank, who evaluated the radiation. Published with permission of the director of the Wisconsin Agricultural Experiment Station. Supported by grants from the Division of Biology and Agriculture, National Research Council.

(2) Tottingham and Lowsma, THIS JOURNAL, **50**, 2436 (1928).

(3) Tottingham, Stephens and Lease, *Plant Physiol*, **9**, 127 (1934).

(4) Ref. 3, p. 130.

(5) Luckiesh, "Artificial Sunlight," D. Van Nostrand Co., New York, 1930, p. 216. We are indebted to the General Electric Company for a loan of such lamps. The Corex glass was donated by the Corning Glass Works.

(6) Tottingham, *Plant Physiol.*, **1**, 328 (1926).

(7) Abbot and Fowle, *Ann. Astrophy. Obsv.*, **2**, Pl. XVI, Curve 11 (1908); Ref. 5, Tab. VI.

(8) Prepared by Dr. R. W. Haman with the use of a Hilger Type E37 quartz spectrograph and Kipp microphotometer.

TABLE I

TOTAL ENERGIES INCIDENT ON PLANTS AND PERCENTAGE DISTRIBUTION THEREOF

Source of radiation microw. per sq. cm.	1000 W. mazda lamp over Corning glass No. 349 and plain glass water cell	500 W. mazda lamp over Corning glass noviol 0 and plain glass water cell	Type S1 sunlight lamp over frosted Corex glass and Corex water cell
Spectral regions, Å.	20,540	20,900	20,900
3100- 4000	0	0.2	5.6
4000- 4500	0	.7	3.6
4500- 5000	0	2.3	2.2
5000- 5500	1.0	3.5	3.4
5500- 6000	4.6	5.0	5.4
6000- 6500	6.6	6.0	4.9
6500- 7000	8.1	7.9	5.8
7000- 7600	11.0	10.9	8.1
7600-14000	68.7	63.5	61.0

from the seedling phase. For proximate analysis the harvested tops were killed at 98° and dried at 55° with ventilation. In the determination of constituents likely to be unstable tissue was analyzed from the fresh state.

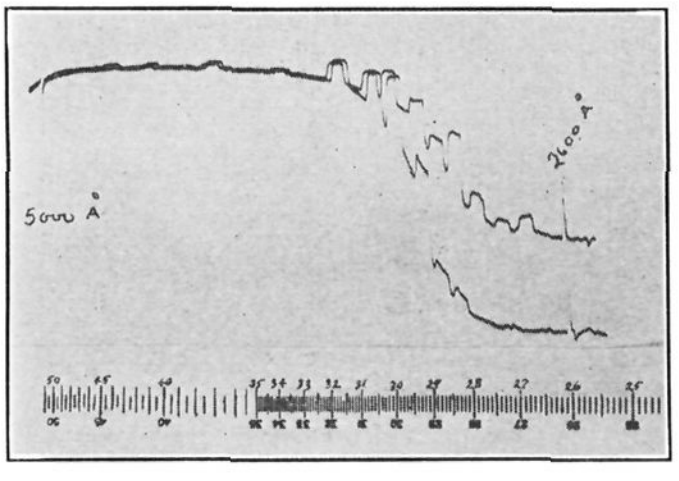

Fig. 2.—Radiation curves of the naked sunlight lamp (upper) and with Corex D water cell and frosted plate interposed (lower).

Tissue Composition.—Proximate analyses of three crops in each case, by standard[9] procedure, gave the average data presented in Tables II and III.

The decline of yield with exposure to shorter wave lengths of light was anticipated from differences in stem diameter, and especially from the stunted form, of plants under the sunlight lamp. There was no external evidence of injury in the latter case but the tissue was characterized by a sharp increase of lipide content and a prominent decline in the percentage of crude fiber. Had this response of lipides depended upon the "wax" content of an increased proportion of plant sur-

(9) Assoc. Offic. Agric. Chem., "Official and Tentative Methods of Analysis," 3d ed., Washington, D. C., 1930.

TABLE II

COMPOSITION OF WHEAT TOPS PRODUCED IN WATER CULTURE

Minimal radiation effective, Å.	Dry matter, g.	Ash, %	Crude fiber, %	Crude fat, %	Crude protein, %	Nitrogen-free extract, %
2900	14.4	21.7	16.9	5.9	30.7	24.8
3900	22.9	21.6	19.3	5.3	27.6	26.2
5200	27.2	21.5	19.5	5.3	26.4	27.3

TABLE III

COMPOSITION OF WHEAT TOPS PRODUCED IN SOIL CULTURE

Minimal radiation effective, Å.	Ash, %	Crude fiber, %	Crude fat, %	True protein, %	Nitrogen-free extract, %
2900	20.4	16.7	5.6	24.9	31.9
3900	19.8	19.1	4.2	23.8	33.1
5200	20.0	19.6	4.1	22.0	34.2

face it should have been much greater. The more available carbohydrates, as represented by the nitrogen-free extract, decreased relatively uniformly with extension of spectral exposure. Protein shows the opposite response, for evidence in Table II indicates that the sharp maximum of crude protein in Table I was probably affected by non-protein nitrogen. Irradiation was without effect upon the proportion of mineral elements in the tissue.

As suggested by the preceding results, the following determinations were selected. Pentosans were determined by the method of McCance[10] on samples prepared by extraction with acetone, ether and hot 80% alcohol. Uronic anhydrides were determined by the procedure of Dickson, Otterson and Link[11] on the extract obtained by boiling with 2.0% hydrochloric acid for two and one-half hours, after extraction of the tissue by ether and water. The first of these constituents was determined on two crops, the second on one. Nitrate and nitrite were determined on fresh tissue by colorimetric procedure,[12] data for the former representing six crops and the latter three. The results appear in Table IV.

TABLE IV

SELECTED DETERMINATIONS ON WHEAT TOPS OF PERCENTAGES IN DRY MATTER

Minimal radiation effective, Å.	Xylan	Uronic anhydride	NO_3	NO_2 (× 1000)
2900	6.9	5.0	1.9	1.3
3900	8.2	4.4	2.2	1.1
5200	9.3	4.0	2.3	1.0

Correlation appears between exposure to ultraviolet and the reduction of nitrate to nitrite. De-

(10) McCance, *Biochem. J.*, **20**, 1111 (1926).

(11) Dickson, Otterson and Link, THIS JOURNAL, **52**, 775 (1930).

(12) (a) Holtz and Larson, *Plant Physiol.*, **4**, 288 (1929); (b) Ref. 9, p. 405.

creases of pentosans and increases of uronic acids are about proportional to the introduction of blue-violet, as shown in Table I, but this does not exclude possible functioning of ultraviolet. Supplementary to the present evidence, we found in another investigation a 20% increase of uronic acid in the wheat stem at prebloom in association with a heavy application of nitrate to the soil. In the present relation this constituent appears to arise as a product of oxidation associated with the reduction of nitrate. It might be expected that ascorbic acid should increase in parallel with uronic acid, but limited titrimetric data obtained by the method of Birch, Harris and Ray[13] indicated very small amounts of this constituent. This situation can be understood on the basis of the observation of Karrer and Bendas[14] that the former constituent reduces nitrite. The results for xylan indicate that the path by which pentosans are formed, commonly supposed to involve decarboxylation of uronic acids, is partially diverted by extensive absorption of nitrates. Press sap from three crops was examined for content of reducing sugars by the Shaffer–Hartman method and for hydrogen ion concentration by use of the quinhydrone electrode. The data, as shown in Table V, are in agreement with those just discussed as indicating increased oxidation of sugars to acids under the influence of shorter visible and longer ultraviolet radiation.

TABLE V

COMPOSITION OF PRESS SAP FROM WHEAT PLANTS THREE WEEKS OLD

Minimal effective radiation, Å.	2900	3900	5200
Reducing sugars, %	2.0	3.2	3.8
*p*H	4.9	5.2	5.4

A search was made for constituents which might serve as trigger mechanisms in the above effects. Several enzyme forms which function in oxidation–reduction relations were inappreciably affected as to activity by the differences in radiation, but it was not feasible to examine dehydrogenases. Three crops of wheat reared under minimal radiation of 2900, 3900 and 5200 Å. gave by titration with potassium iodate according to the procedure of Okuda and Ogawa[15] relative proportions in glutathione of 100, 91 and 68, respectively. Similar results were obtained for the sulfhydryl group in tomato leaves irradiated for three hours daily by the sunlight lamp as a supplement to July sunlight under glass. Six samples showed an increase of about 100% by this exposure as compared with supplementary use of the mazda lamp as a control. In this case the method of Guthrie[16] was applied, excepting that the alcoholic extract was inactive and the whole tissue was exposed to reaction with colloidal sulfur. In view of the absorptive capacity of cystine for both blue-violet and ultraviolet light, as observed by Ward,[17] it is suggestive to find here a reduction product of that form of sulfur. Szent-Gyorgyi[18] has shown that glutathione reduces hexuronic acids, a relation which might confer upon sulfhydryl compounds in the present instance significance in the utilization of sugars for nitrate assimilation under shorter rays of light.

Some attention was devoted to the leaf pigments. Six samples gave the average data of Table VI, by colorimetric procedure.

TABLE VI

PERCENTAGES OF PIGMENTS IN LEAF DRY MATTER OF WHEAT PLANTS 25 DAYS OLD

Minimal effective radiation, Å.	Chlorophylls	Carotenes	Xanthophylls
2900	0.74	0.10	0.19
5200	.61	.09	.18

The lesser content of chlorophylls where shorter radiations were excluded could be anticipated by color differences of the leaves. It agrees with previous observations[19] of ineffectiveness of red and infra-red rays in synthesis of these pigments. This increase of pigment in plants receiving the more complete spectrum may be highly significant in relation to increased nitrate assimilation. Substantial physiological information indicates that it could function through stimulating respiration,[20] the latter process in turn providing for reduction of nitrate.[21]

Large-scale lipide preparations were recovered by ether from plants exposed to April sunlight under glass, supplemented by the use of either mazda or sunlight lamps. These were found to have the same proportion and character of wax fraction under the differences of radiation. Fatty acids recovered from the non-waxy fraction were

(13) Birch, Harris and Ray, *Biochem. J.*, **27**, 590 (1933).

(14) Karrer and Bendas, *Helv. Chim. Acta*, **17**, 743 (1934).

(15) Okuda and Ogawa, *J. Biochem.*, **18**, 75 (1933).

(16) Guthrie, *Contrib. Boyce Thomp. Inst.*, **4**, 99 (1932).

(17) Ward, *Biochem. J.*, **17**, 898 (1923).

(18) Szent-Gyorgyi, *ibid.*, **22**, 1399 (1929).

(19) Sayre, *Plant Physiol.*, **3**, 71 (1928); Johnston, Smithson, *Misc. Coll.*, **87**, No. 14 (1932).

(20) Spoehr and McGee, Carnegie Inst. Wash., Pub. 325, 76 (1923).

(21) Warburg and Negelein, *Biochem. Z.*, **110**, 66 (1920).

somewhat less saturated under the sunlight lamp than under the mazda, with iodine numbers of 106.6 and 103.9, respectively. Sterols were recovered as digitonides to the extent of 37 and 34 mg. per 100 mg. of dry tissue in these respective samples. These data supply limited evidence of increased dehydrogenation under the higher proportion of short-wave radiation.

Summary

Elimination of wave lengths shorter than about 3900 or 5200 Å. from radiation resembling sunlight decreased the assimilation of nitrate and conserved carbohydrates in young wheat plants. The primary factors to which increased reducing power under shorter radiation could be attributed were increased tissue contents of chlorophylls and sulfhydryl compounds. Paths are indicated by way of which these factors could function as trigger mechanisms in the reduction of nitrate. Depression of pentosan formation was associated with increased assimilation of nitrate. Most of the compositional effects in the tissue could be attributed to variations in the proportion of blue-violet light, but the reduction of nitrate to nitrite was more distinctly associated with long ultraviolet radiation. Apparently the high proportion in sunlight of radiation from 3900 to 4920 Å. and the low proportion above 8000 Å., in comparison with the light sources here tested, favor the assimilation of nitrate by plants.

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The Dimorphism of Rotenone

BY ERNEST L. GOODEN AND CHARLES M. SMITH

The fact that rotenone occurs in two forms characterized by melting points of 163° and about 180° was briefly referred to by Butenandt and Hildebrandt[1] and later considered more fully by Cahn.[2] This phenomenon has also been studied in the Insecticide Division and, since certain characteristics of the two materials not previously recorded have been determined, the results of this study are given here.

No attempt has been made to confirm the statement of Butenandt and Hildebrandt that the higher melting form of rotenone can be prepared from the ordinary form by grinding, but the transition during melting-point determinations and the melting and resolidification of rotenone that was rapidly heated to a temperature between the two melting points have been observed. Neither of these two methods of preparation gives a product suitable for microscopical study, but the higher melting material can be prepared easily in well-crystallized form by the process given in the following section.

Preparation of Materials

The sample of low-melting rotenone was obtained from cubé root, being crystallized once from carbon tetrachloride as the solvate, and then twice from a 1:1 mixture of acetone and water as rotenone itself.

The high-melting rotenone was prepared from this sample by immersing it in a petroleum oil less volatile than kerosene and heating to 140–150°. The mixture was maintained in this temperature range, with frequent stirring, at least until examination with a polarizing microscope showed apparently complete transformation of the original thin six-sided plates to chunky, granular crystals.[3] The petroleum oil was decanted, and the crystals were washed by decantation with petroleum ether and dried at room temperature. Four batches were prepared in this manner, with different periods of heating, lasting from one to five hours. None of the batches was pure white, as it is presumed they should have been, but the one heated least was the best looking preparation and was considered most nearly pure.

That this material was not a solvate was proved by microanalysis[4] and a determination of the opti-

(1) Butenandt and Hildebrandt, *Ann.*, **477**, 245 (1930).

(2) Cahn, *J. Chem. Soc.*, 1129 (1934).

(3) No method of proving the completeness of the transformation was devised. The writers feel certain that it was nearly enough complete to demonstrate a difference in density if any considerable difference existed. The optical data are, of course, not affected by this uncertainty, for they were determined on individual crystals known to be of the high-melting form.

(4) The writers wish to thank Dr. J. R. Spies for determinations of carbon and hydrogen.