14. VICKERY, H. B. The metabolism of the organic acids of tobacco leaves. VIII. Effect of culture of excised leaves in solutions of L-malate at pH 4 to pH 7. Jour. Biol. Chem. 214: 323–333. 1955.
15. VICKERY, H. B., PUCHER, G. W., WAKEMAN, A. J. and LEAVENWORTH, C. S. Chemical investigations of the tobacco plant. VI. Chemical changes that occur in leaves during culture in light and in darkness. Conn. Agr. Expt. Station. Bull. 399. 755–832. 1937.
16. VICKERY, H. B. The formation of starch in leaves of *Bryophyllum calycinum* cultured in darkness. Plant Physiol. 27: 231–239. 1952.
17. VICKERY, H. B. The formation of starch and the behavior of isocitric acid in excised leaves of *Bryophyllum calycinum* cultured in darkness. Plant Physiol. 32: 220–224. 1957.
18. VICKERY, H. B. The effect of temperature on the behavior of malic acid and starch in leaves of *Bryophyllum calycinum* cultured in darkness. Plant Physiol. 29: 385–392. 1954.
19. VICKERY, H. B. and WILSON, D. G. Preparation of potassium dihydrogen L_s (+)-isocitrate from *Bryophyllum calycinum* leaves. Jour. Biol. Chem. 233: 14–17. 1958.
20. WOLF, J. Beiträge zur Kenntnis des Säuerstoffwechsels sukkulenter Crassulacean. IV. Beobachtungen über Gehaltsschwankungen von Gesamt-, Äpfel- und Zitronensäuren. Planta. 29: 314–324. 1939.
21. ZBINOVSKY, V. and BURRIS, R. H. Metabolism of infiltrated organic acids by tobacco leaves. Plant Physiol. 27: 240–250. 1952.

RESPIRATORY AND ASSOCIATED RESPONSES OF CARROT DISCS TO SUBSTITUTED PHENOLS [1]

B. K. GAUR [2] AND HARRY BEEVERS
DEPARTMENT OF BIOLOGICAL SCIENCES, PURDUE UNIVERSITY, LAFAYETTE, INDIANA

The striking physiological and biochemical effects of the classical uncoupling agent 2,4-dinitrophenol (DNP) are now well known, (4, 5, 15) though still imperfectly understood. The pertinent work with plant materials has been reviewed by Simon (21) and Laties (16). Stenlid, who carried out some of the earliest work on plants, reported that 5 other nitrophenols brought about similar respiratory responses to those induced by DNP, and, like DNP, inhibited glucose uptake by wheat roots (24). The behavior of the nitrophenols was compared to that of azide (23) and later to that of methylene blue (25).

In this paper quantitative comparisons have been made of the effects of a series of NO_2-, Cl-, and Br-substituted phenols on respiration and concommitant glucose uptake. In addition, the effects of these compounds on mitochondrial oxidations have been determined. The conclusion is drawn that, with different degrees of efficiency, all of the substituted phenols acted as uncoupling agents.

Observations on the effects of the phenols on $C^{14}O_2$-output from glucose-C^{14} are also presented and discussed in relation to the question raised recently by Porter and Runeckles (19) of a possible shift in respiratory substrate resulting from DNP treatment.

MATERIALS AND METHODS

Carrots of 25 to 30 mm average diameter were purchased locally and stored at 3 to 5° C for at least a week before use. Discs of about 0.3 mm thickness and 7 mm diameter were prepared, washed quickly in 3 changes of distilled water, dried lightly, and separated into weighed samples. The various phenols and other reagents were of the best grade commercially available, and were used without further purification. Radioactive glucose, uniformly labeled with C^{14} was prepared by hydrolysis and chromatographic separation from a sample of sucrose-C^{14} produced during photosynthesis in $C^{14}O_2$ and generously supplied by Dr. H. R. Garner. All determinations of radioactivity were carried out in a gas flow windowless counting system, and the results are corrected for background, and in the case of carbonate, for self absorption.

Two types of experiments were carried out. In the 1st, the aim was to determine the relative effects on O_2-uptake of a group of phenols and other compounds, by testing responses over a wide range of concentrations. For such experiments 650 mg samples of discs were placed in Warburg flasks (with the test compound where necessary) in 2 ml 0.03 M potassium phosphate pH 5.0. O_2 uptake was measured over a 2-hour period in the usual manner at 25° C.

The information from these experiments was used to choose, for each compound, concentrations which were: a) slightly stimulatory, b) strongly stimulatory, and c) inhibitory to O_2-uptake. In the 2nd series of experiments the test compounds were supplied at each of these 3 levels and stimultaneous measurements of O_2-uptake and glucose uptake were made over a longer experimental period (6 to 8 hr). In these experiments 2 series of flasks were assembled. The 1st was composed of large vessels (100 ml capacity) and these contained 1.30 g discs, the test compound, and an aliquot of radioactive glucose (0.036 mg) (tipped from the side arm after equilibration) in 4 ml

[1] Received revised manuscript November 3, 1958.
[2] Present address: Biology Division,, A. E. E. T., Indian Cancer Research Centre, Parel, Bombay, India.

0.03 M phosphate pH 5.0. In order to measure glucose uptake, samples (0.1 ml, containing initially about 400 cpm) were removed at hourly intervals from these large flasks. These were dried under an infrared lamp on steel planchets and counted directly. The decrease in radioactivity was used to calculate glucose uptake.

The 2nd, parallel, series was made up of small flasks each containing 650 mg discs in 2 ml of solution with proportionate amounts of glucose and the test compounds. These flasks were used to obtain a continuous record of O_2-uptake of controls and treated samples during the experiment; they were not opened when samples were removed from the larger flasks. The respired CO_2 was trapped in 20 % sodium hydroxide and the radioactivity determined at the end of the experiment by converting to barium carbonate, plating and counting. For each of the compounds tested an experiment of this kind was carried out and then repeated at least 3 times. The results were then pooled and averaged. In the experiments with mitochondria, castor beans were used; the methods were those described by Beevers and Walker (3).

Results

A. Experiments in Which Respiration Only Was Measured: When different concentrations of 2,4-dinitrophenol were applied, the anticipated stimulatory and inhibitory effects were established from the outset, and O_2-uptake continued unchanged at these new levels throughout the period of measurement. From data obtained in this way dosage response curves were constructed for each compound.

The forms of the various response curves were similar; for each compound only a rather narrow range of concentrations induced stimulated O_2-uptake, and inhibitory effects ensued when higher levels were applied. The response curves differed from each other in 2 respects; the extent of the stimulation induced at the most effective levels of the test compound and in the placing of the response curve along the concentration axis.

We may note from table I that on both of these counts unsubstituted phenol is the least effective compound; not only is the maximum stimulation a mere 20 % above the control, but the concentration required to induce this is considerably greater than 0.01 M. By contrast, dinitrophenol, the most effective compound, elicited a maximum response (236 % of the control rate) at a concentration 0.000025 M (table I).

Between these extremes lie the responses to the other compounds tested. The single substitution of Cl-, Br-, or NO_2-, in any position results in a compound greatly superior to phenol itself in its stimulatory ability. *Para*-substitution was particularly effective in all 3 classes, and resulted in stimulation as striking as those from dinitrophenol and dichlorophenol. However, the concentrations which were required to induce these effects were somewhat higher than the corresponding dinitrophenol level. These differences are shown in table I, in which the effectiveness of each compound in inducing stimulated respiration is related to that of dinitrophenol by the calculation of a DNP index.

For comparison, results obtained with azide and with methylene blue are included. In no case was azide found to induce a significant stimulation of respiration, methylene blue was effective over a rather wide range at high concentrations.

B. Experiments in Which Glucose Uptake and O_2-uptake Were Measured: The experimental design outlined above allowed the following

Table I

Effects of Various Compounds on the Oxygen Uptake of Carrot Discs

Compound	Maximum stimulation observed	Concentration inducing maximum stimulation	Concentration inducing 50 % inhibition	DNP index *
	% of Controls	$M \times 10^{-5}$	$M \times 10^{-5}$	
Phenol	120	2500	6600	1000
o-Nitrophenol	155	250	1000	100
m-Nitrophenol	227	250	580	100
p-Nitrophenol	242	60	480	25
2,4-Dinitrophenol	236	2.5	20	1
o-Chlorophenol	180	760	1400	301
m-Chlorophenol	160	125	680	50
p-Chlorophenol	241	125	680	50
2,4-Dichlorophenol	253	60	160	25
o-Bromophenol	200	76	630	30
m-Bromophenol	146	76	400	30
p-Bromophenol	232	76	400	30
Methylene blue	170	50	1600	20
Sodium azide	No stimulation from 10^{-8} M—3 $\times$ 10^{-5} M		5	...

$$\text{* DNP index} = \frac{\text{Molar concentration of test compound giving maximum stimulation}}{\text{Molar concentration of 2,4-dinitrophenol giving maximum stimulation}}$$

determinations to be made: a) a continuous record of O_2-uptake b) hourly progress of glucose uptake and c) total $C^{14}O_2$ release, determined at the end of the experiment. From this primary data the information contained in table II was obtained. The effects of the test compounds on O_2-uptake, glucose uptake, and $C^{14}O_2$ output are recorded as percentages (table II) of the corresponding control rates. As indicated at the foot of table II the control values observed in the several experiments are quite uniform. They show that the bulk of the supplied glucose was absorbed during each experiment and that only a small fraction of this was converted to $C^{14}O_2$. The responses to the various test compounds at each of the 3 chosen concentrations are shown in table II. Again a uniform pattern emerges.

The O_2-uptake figures show firstly that the anticipated respiratory responses were induced, although it should be noticed that the stimulations are not, in general, so large as those observed in the corresponding short term experiments. Strong inhibitions of glucose uptake are induced by concentrations of the phenols which curtail O_2-uptake, but what is more important, striking inhibitions are obtained at those levels which are most effective in stimulating respiration, and correspondingly smaller effects, again inhibitory ones, are obtained at still lower levels of the test compounds, which elicit smaller respiratory stimulations. The values in table III emphasize one important aspect of the results, that all of the test compounds, at levels which reduced glucose uptake by half, induced stimulations in the rate of O_2-uptake (table III).

For each substituted phenol the curves relating its concentration to its effects on glucose uptake and O_2-uptake, diverged in opposite directions from the horizontal when a particular concentration was exceeded. Thus in their relative effectiveness as inhibitors of glucose uptake the various phenols are related to dinitrophenol in the same way as they were found to be in the respiration experiments. That is to say, dichlorophenol, *p*-nitrophenol, and the *m*- and *p*-substituted chloro- and bromo-phenols were 20 to 50 times less potent than dinitrophenol, and the *o*-substituted phenols were the least effective.

It will be seen that in a few instances (*m*- and *p*-substituted nitro- and chloro-phenols) in table II, glucose uptake was apparently less strongly inhibited at the highest concentration of phenol than it was at the intermediate level. It was noticed that in those examples a considerable amount of "leakage" had occurred from the slices during the experiment so that on drying down the aliquots removed for counting a lacquer-like deposit was obtained, whereas normally only a very thin film was obtained on drying. The

TABLE II

SIMULTANEOUS OBSERVATIONS ON THE UPTAKE OF OXYGEN AND RADIOACTIVE GLUCOSE BY CARROT DISCS IN RESPONSE TO SUBSTITUTED PHENOLS

COMPOUND	LOG. MOLAR CONCENTRATION	OXYGEN UPTAKE	SUGAR UPTAKE	$C^{14}O_2$ EVOLVED
		% of controls *		
o-Nitrophenol	−3.2	108	89	84
	−2.6	130	26	49
	−2.2	70	3	5
m-Nitrophenol	−3.3	110	84	101
	−2.6	117	6	3
	−2.2	21	18	2
p-Nitrophenol	−3.3	168	36	47
	−3.1	91	14	16
	−2.3	6	37	1
2,4-Dinitrophenol	−5.6	160	88	96
	−4.6	220	44	51
	−3.6	14	17	2
o-Chlorophenol	−3.2	116	58	61
	−2.9	128	35	27
	−2.6	144	26	9
m-Chlorophenol	−3.2	113	30	31
	−2.9	137	15	9
	−2.3	73	25	1
p-Chlorophenol	−3.4	181	39	63
	−2.9	185	6	7
	−2.2	4	35	1
2,4-Dichlorophenol	−4.3	123	86	81
	−3.2	195	10	9
	−2.9	37	2	3
o-Bromophenol	−4.3	110	96	89
	−3.3	117	76	86
	−2.3	110	18	6
m-Bromophenol	−4.3	106	100	101
	−3.1	115	25	19
	−2.6	71	22	25
p-Bromophenol	−4.3	107	96	93
	−3.3	150	35	53
	−2.6	47	17	1

* The control samples in the above experiments took up from 76 to 93 % (average 84 %) of the supplied glucose-C^{14} and converted 8.1 to 11.9 % (average (10.0 %)) of this to $C^{14}O_2$.

TABLE III

RESPIRATORY RESPONSES TO SUBSTITUTED PHENOLS IN CONCENTRATIONS WHICH INHIBITED GLUCOSE UPTAKE BY 50 %

COMPOUND	CONCENTRATION AT WHICH GLUCOSE UPTAKE WAS INHIBITED BY 50 %. $M \times 10^{-5}$	RESPIRATION AT THIS CONCENTRATION (O_2-UPTAKE AS % OF CONTROLS)
o-Nitrophenol	159	123
m-Nitrophenol	87	116
p-Nitrophenol	42	167
2,4-Dinitrophenol	2	218
o-Chlorophenol	79	120
m-Chlorophenol	25	105
p-Chlorophenol	23	170
2,4-Dichlorophenol	19	158
o-Bromophenol	178	113
m-Bromophenol	32	115
p-Bromophenol	27	138

effect of the materials lost from the slices would be to increase self absorption and so to give a lower counting rate. This would lead to a spuriously high value for glucose uptake. We do not feel justified therefore in attaching significance to the apparently lower effectiveness of the highest concentrations of some of the test compounds.

The figures for $C^{14}O_2$ output during the experiments with glucose-C^{14} are shown in the final column of table II. It should be emphasized that, of the total glucose absorbed by the controls, only about 10 % was converted to CO_2. Nevertheless it is clear that the test compounds drastically reduced the production of $C^{14}O_2$ at whatever level they were applied. The very close parallel between the responses of $C^{14}O_2$ production and glucose uptake to individual concentrations of the test compound should be especially noted.

C. Experiments with Mitochondria: The responses of plant mitochondria to DNP have been described by several authors (1, 6, 16, 17, 18) and it is clear that P/O ratios are drastically reduced. These experiments have usually been carried out in the presence of the hexokinase-glucose trapping system, which, although it allows the inhibitory effect of DNP on phosphate esterification to be shown, may preclude the demonstration of what is an equally distinctive effect, namely that of stimulated O_2-uptake. In castor bean mitochondria such a stimulatory effect of DNP has been described, (1), and we have now investigated the ability of other substituted phenols to bring about this response. For each compound a series of concentrations was applied and response curves were constructed from the results of triplicate experiments. The values in table IV were read off from the curves (table IV). Striking stimulations were induced by all of the phenols; again the di-substituted phenols were particularly effective, with the *p*-, *m*-, and *o*-substituted phenols following in that order. Methylene blue induced a considerable stimulation while azide had virtually no such effect. Inhibitions of O_2-uptake ensued when the concentrations of all the compounds were raised above the stimulating one.

Table IV

Effects of Various Substituted Phenols, Sodium Azide and Methylene Blue on the Oxygen Consumption by Mitochondria from Castor Bean Endosperm *

Test compound	Maximum stimulation	Molar conc giving maximum stimulation	Molar conc giving 50 % inhibition
	% *of control*	*M* × 10^{-3}	*M* × 10^{-3}
o-Nitrophenol	184	5.0	9.8
m-Nitrophenol	205	1.3	5.5
p-Nitrophenol	204	0.4	5.3
Dinitrophenol	204	0.3	6.3
o-Chlorophenol	146	3.7	12.0
m-Chlorophenol	141	2.5	4.9
p-Chlorophenol	189	2.5	7.6
Dichlorophenol	182	0.5	2.8
Sodium azide	107	0.5	3.1
Methylene blue	145	0.3	47.9

* The mitochondria were prepared as described by Beevers and Walker and O_2-uptake was measured over a 30-minute period with α-ketoglutarate (0.005 M) as substrate (3).

Discussion

It is clear from the results that all of the substituted phenols, when applied at appropriate concentrations have the following properties in common with the classical uncoupling agent DNP: a) They stimulate the respiration of carrot discs; b) at levels stimulatory to O_2-uptake, they inhibit the associated endergonic process of glucose uptake; and c) they stimulate the O_2-uptake of castor bean mitochondria oxidizing α-ketoglutarate.

It is concluded that such compounds, like DNP, are uncoupling agents.

From their different abilities to induce the responses described (tables I, II and IV), the following conclusions were drawn about the relative effectiveness of the various phenols. 2,4-dinitrophenol was the most effective compound, i.e., it induced responses at the lowest levels. This was followed in turn by 2,4-dichlorophenol, and the *para*-substituted nitro-, chloro- and bromo-phenols; the *ortho*-substituted compounds were less effective but considerably more active than phenol itself. The responses to the addition of various substituents in the molecule thus conform in general to a pattern which has emerged from more extensive comparative work with other materials, in which usually only one response, i.e., a respiratory effect or an inhibitory action, has been measured. Among this work may be mentioned particularly that of Stenlid on wheat roots (24), Clowes and Krahl (7, 14) on sea urchin eggs, of Simon and Blackman (20, 22) on *Trichoderma viride*, *Lemna minor* and *Brassica alba* and of Field, Martin and Field (9) and more recently Deken (8) on yeast. We suppose, with the previous authors, that the differences in effectiveness most probably reflect, to a large degree, the ease of penetration of the phenols to the points of primary attack, which, one would now suppose, are the enzymes in the mitochondria concerned with electron transfer.

Azide failed to induce respiratory stimulations in the experiments reported here, although effects which can be reasonably ascribed to an uncoupling action have been described in plants by Stenlid (23) and by Harley and his colleagues (12). Presumably respiratory stimulations can only be demonstrated if the level of azide required is lower than that at which the oxidation enzymes are themselves inhibited. As in Stenlid's experiments (25) methylene blue induced effects on respiration and glucose uptake which were similar to those due to the phenols. This parallelism has now been shown to extend to the mitochondria. It should be noticed however, that the mechanism of action of methylene blue may be rather different from that of the phenols, since it is itself autooxidizable

and may effectively by-pass normal electron transfer steps (13).

In considering the $C^{14}O_2$ results it should first be emphasized that the carrot tissue contains ample available carbohydrate reserves, and its respiration, with a respiratory quotient close to unity is not increased by the small amounts of glucose supplied. At all levels of DNP (and, by inference, of the other phenols) which stimulate O_2-uptake, CO_2-output is stimulated to at least the same extent (2). But, at these stimulatory levels, as shown in table III $C^{14}O_2$ output was drastically reduced, so that the specific activity of the respired CO_2 declines with increasing DNP. Such an observation was one of the arguments used by Porter and Runeckles (19) in support of an otherwise well founded suggestion that in tobacco leaves DNP induced the respiration of non-carbohydrate material. However, the final 2 columns in table II show that there is a striking parallelism between the inhibitory effects of each phenol on glucose uptake and on $C^{14}O_2$ release. In the carrot tissue then, it is possible to ascribe the lowered specific activity of the CO_2 directly to a reduced uptake of the radioactive glucose, and it is not necessary to invoke a change in respiratory substrate as an explanation. It should be noted that in contrast to the carrot tissue used in these experiments and those described previously (2) the tobacco leaf tissue had a low RQ (0.7), its respiration was strikingly stimulated by the added sugar, and the experiments were carried out in 100 % O_2. Whatever may be the explanation of the responses to DNP in the tobacco tissue, the present results confirm that no change is necessary in the conclusions from our previous experiments, (2, 10), namely that the respiratory responses to DNP in the tissues used can be ascribed to an increased rate of glycolysis brought about as an indirect result of the uncoupling action at the electron transfer level.

Summary

The effects of a series of substituted phenols on O_2-uptake and glucose accumulation by carrot discs have been determined. The response to each compound was a respiratory stimulation, which reached a maximum and then declined sharply to an inhibition as the concentration was raised over a rather narrow range. The striking changes in respiration as the concentrations were increased were accompanied by the progressive inhibition of glucose absorption; those concentrations which induced maximally stimulated O_2-uptake drastically curtailed glucose uptake. The various phenols induced stimulations of O_2-uptake by castor bean mitochondria which were similar to those induced by dinitrophenol. The various substituted phenols were thus supposed to be acting, with varying degrees of efficiency, as uncoupling agents.

The relative effectiveness was gauged by comparing the concentrations of the compounds required to induce maximally stimulated respiration, which in most instances amounted to over 200 % of the control rates. 2,4-Dinitrophenol was the most effective compound, followed, in turn by 2,4-dichlorophenol, *p*-nitrophenol, the bromophenols, *m*- and *p*-chlorophenol, *m*- and *o*-nitrophenol, *o*-chlorophenol and phenol itself, which induced at best only 20 % stimulation.

In parallel experiments with methylene blue it was shown that effects similar to those of the substituted phenols were induced. Azide induced only respiratory inhibitions and corresponding curtailment of glucose accumulation in carrot discs.

It was also demonstrated that the appearance of radioactive carbon in the respired CO_2 was inhibited only to the same degree as the uptake of the glucose-C^{14} was itself prevented. For this reason the effects of substituted phenols on $C^{14}O_2$ production cannot be considered as evidence for a changeover in respiratory substrate in response to their addition.

This work has been supported in part by the Atomic Energy Commission, (Contract no. AT-(11-1)-330). We record our appreciation of this support.

Literature Cited

1. Akazawa, T. and Beevers, H. Mitochondria in the endosperm of the germinating castor bean: a developmental study. Biochem. Jour. 67: 115–118. 1957.
2. Beevers, H. 2,4-Dinitrophenol and plant respiration. Amer. Jour. Bot. 40: 91–96. 1953.
3. Beevers, H. and Walker, D. A. The oxidative activity of particulate fractions from germinating castor beans. Biochem. Jour. 62: 114–120. 1956.
4. Bonner, J. Limiting factors and growth inhibitors in the growth of *Avena coleoptile*. Amer. Jour. Bot. 36: 323–332. 1949.
5. Bonner, J. Relations of respiration and growth in *Avena coleoptile*. Amer. Jour. Bot. 36: 429–436. 1949.
6. Bonner, J. and Millerd, A. Oxidative phosphorylation by plant mitochondria. Arch. Biochem. Biophys. 42: 135–148. 1953.
7. Clowes, G. H. A. and Krahl, M. E. Studies on cell metabolism and cell division. I. On the relation between molecular structures, chemical properties and biological activities of nitrophenols. Jour. Gen. Physiol. 20: 145–171. 1936.
8. Deken, de R. H. Relations entre la structure moleculaire et le pouvoir inhibiteur des nitro et halophenols. Biochim. Biophys. Acta 17: 494–502. 1955.
9. Field, J., Martin, A. W. and Field, S. M. The effect of 2,4-dinitrophenol on the oxygen consumption of yeast. Jour. Cellular Comp. Physiol. 4: 405–420. 1934.
10. French, R. C. and Beevers, H. Respiratory and growth responses induced by growth regulators and allied compounds. Amer. Jour. Bot. 40: 660–666. 1953.
11. Gaur, B. K. Some effects of uncoupling agents on plant tissues and mitochondria. Ph.D. thesis, Purdue University, Lafayette 1957.
12. Harley, J. L., McCready, C. C., Brierley, J. K. and Jennings, D. H. The salt respiration of excised beech mycorrhizas. II. The relationship be-

tween oxygen consumption and phosphate absorption. New Phytologist 55: 1–28. 1956.
13. JUDAH, J. D. and WILLIAMS-ASHMAN, H. G. The inhibition of oxidative phosphorylation. Biochem. Jour. 48: 33–42. 1951.
14. KRAHL, M. E. and CLOWES, G. H. A. Studies on cell metabolism and cell division. II. Stimulation of cellular oxidation and reversible inhibition of cell division by dihalo and trihalophenols. Jour. Gen. Physiol. 20: 173–184. 1936.
15. LARDY, H. A. and WELLMAN, H. Oxidative phosphorylations: role of inorganic phosphate and acceptor systems in control of metabolic rates. Jour. Biol. Chem. 195: 215–244. 1952.
16. LATIES, G. G. The physical environment and oxidative and phosphorylative capacities of higher plant mitochondria. Plant Physiol. 28: 557–575. 1953.
17. LATIES, G. G. Respiration and cellular work and the regulation of the respiration rate in plants. Survey of Biol. Progress. 3: 215–299. 1957.
18. LIEBERMAN, M. and BIALE, J. B. Oxidative phosphorylation by sweet potato mitochondria and its inhibition by polyphenols. Plant Physiol. 31: 420–424. 1956.
19. PORTER, H. K. and RUNECKLES, V. C. Some effects of 2,4-dinitrophenol upon the metabolism of tobacco leaf discs. Biochim. Biophys. Acta 20: 100–108. 1956.
20. SIMON, E. W. The action of nitrophenols on respiration and on glucose assimilation in yeast. Jour. Expt. Bot. 4: 377–392. 1953.
21. SIMON, E. W. Mechanisms of dinitrophenol toxicity. Biol. Reviews 28: 453–479. 1953.
22. SIMON, E. W. and BLACKMAN, G. E. Studies on the principles of phytotoxicity. IV. The effects of the degree of nitration on the toxicity of phenol and other substituted benzenes. Jour. Expt. Bot. 4: 235–250. 1953.
23. STENLID, G. Some notes on the effect of sodium azide, dinitrophenol and *o*-phenanthroline upon oxygen consumption in green leaves. Physiol. Plantarum 2: 61–69. 1949.
24. STENLID, G. The effect of 2,4-dinitrophenol upon oxygen consumption and glucose uptake in young wheat roots. Physiol. Plantarum 2: 350–371. 1949.
25. STENLID, G. Methylene blue and α-α^1 dipyridyl, two different types of inhibitors for aerobic metabolism in young wheat roots. Physiol. Plantarum 3: 197–203. 1950.

STUDIES ON THE MECHANISM OF HERBICIDAL ACTION OF 2-CHLORO-4,6-BIS(ETHYLAMINO)-S-TRIAZINE [1, 2]

D. E. MORELAND [3], W. A. GENTNER [4], J. L. HILTON [4] AND K. L. HILL [3]
NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION AND U. S. D. A., RALEIGH, NORTH CAROLINA

Recent studies have shown that certain triazine derivatives can be used to control weeds in specific crops at relatively low rates of application (2, 3). At higher rates of application they can be used as soil sterilants for general weed control on non-cropped areas. One of these compounds, 2-chloro-4,6-bis-(ethylamino)-s-triazine [simazin] [5], applied as a pre-emergent spray at 2 to 4 pounds per acre has given excellent control of annual weeds in corn for practically the entire growing season (3). Corn appears to be especially tolerant to the chemical and very high rates of application have been used successfully in some studies. Barley and other small grains are not able to tolerate pre-emergent applications of even 2 pounds per acre of simazin (2, 3).

The objectives of the studies reported herein were a) to determine the effects of simazin on the photochemical activity of chloroplasts isolated from barley plants and b) to determine whether the inhibitory effects of simazin could be overcome by supplying treated barley plants with carbohydrates from an external source. A preliminary report (4) of these findings has been presented.

MATERIALS AND METHODS

STUDIES WITH ISOLATED CHLOROPLASTS: The chloroplasts used in this study were obtained from 10-day-old greenhouse-grown barley plants (*Hordeum vulgare* L. variety Dayton, CI 9517) using the procedure given by Spikes et al (6). Following the initial isolation they were washed 3 times with 0.5 M sucrose.

The photochemical activity of the isolated chloroplasts was measured in small beakers. A 10-ml reaction mixture was used which had the following composition: 0.0005 M potassium ferricyanide, 0.01 M potassium chloride, 0.17 M sucrose, 0.05 M potassium

[1] Received November 24, 1958.
[2] Cooperative investigations of the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture and the North Carolina Agricultural Experiment Station. Published with the approval of the North Carolina Agricultural Experiment Station as Paper no. 933.
[3] Plant Physiologists, Agricultural Research Service, U. S. Department of Agriculture, N. C. State College, Raleigh, N. C.
[4] Plant Physiologists, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.
[5] The experimental samples used in these studies were supplied by personnel of the Geigy Chemical Corporation, Ardsley, New York.