Lectures on

Soil Organic Matter

by

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Foreword

To my friends and the friends of the subject.

In this manuscript are some unpublished results and therefore only for friends and not to use for publication.

I would enjoy having any comments on this material.

W. Flaig

Acknowledgments

This manuscript came about as a result of the kind invitation of Prof. Dr. W. H. Pierre, Head, Department of Agronomy, to give lectures about soil biochemistry. I am very thankful for this opportunity.

I would like to express my best thanks also to my colleague, Prof. Dr. Lloyd Frederick, who stood by me helpfully at all times during the writing of these lectures in the English language and I appreciate his suggestions during our many discussions.

Without the help of his co-workers, Messrs. McIntosh Sims, Horton, Brown, and of the secretaries, Mrs. McLaughlin, Misses Sansgaard and Zart, it would not have been possible to mimeograph the lectures. Also to these, many thanks.

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W. Flaig

SOIL ORGANIC MATTER

Table of Contents

Physiological Effect on Plant Growth

(Supplement to the lecture before the Agronomy Society)

In connection with investigations about the influence of organic substances of the soil, especially of the group of humic substances, on the metabolism of plants, we use also quinones as model substances. Decomposition products of the humic acids isolated from the soil and chemical investigations about the transformation of lignin into humic substances were the reason for this.

S 524

Table 1. Influence on germination and growth of germinated plants of grain (cereals) by thymohydroquinone.

conc.-thymo-hydro-quinone	number of germinated grains				sprout weights in gram			
	wheat		rye		wheat		rye	
	abs.	rel.	abs.	rel.	abs.	rel.	abs.	rel.
0	52±1.3	100	75±1.3	100	1.54±0.06	100	0.82±0.04	100
$1.5.10^{-3}$m	0	0	0	0	0.86±0.03	57	0	0
$1.5.10^{-4}$m	52±3.2	100	67±0.5	89	1.60±0.02	104	0.98±0.02	114
$1.5.10^{-5}$m	64±2.7	123	86±1.3	115	1.69±0.04	110	1.05±0.01	128
$1.5.10^{-6}$m	55±2.8	106	85±1.7	114	1.61±0.01	104	1.00±0.03	121

During the course of the investigations with model substances we found out that thymoquinone and thymohydroquinone increase germination (1) and that the addition to nutrient solutions in concentrations between 10^{-4} and 10^{-6} mol/L also increased the growth and yield of fresh and dry substance (2) of cereals. A higher germination and an increasing of the yield were also found in the field experiment (3).

To keep the connection to the investigations about the humic substances, the relation between the effectiveness of different substituted quinones or hydroquinones and their chemical constitution was investigated. These experiments were expanded furthermore with fractions of the humic substances and with decomposition products of lignin not only on the initial growth but also on to the total vegetation production in pot experiments.

Without regard to the dependency of the effect of the kind of substitution, position and number of the substituents, it was found that the quinones and hydroquinones of same substitution differ in that the quinones in general are already effective in lower concentration than the corresponding hydroquinones, though these are mostly more easily soluble. To explain the different effect, not only the chemical but also the physical properties of the substances seem to be important, since these determine the assimilation by the plant and the transport to the points of effectiveness. These two processes must take place through complicated mechanisms, otherwise it would not be understandable that the more difficultly soluble quinones in a lower concentration are more effective than the more easily soluble hydroquinones.

After it had been found that the applied quinones and hydroquinones used as model substances are assimilated through the plants and influence the plant growth directly, it was of interest to analyze their effect on metabolism in detail.

Indications about the points of effectiveness resulted from experiments about the initial growth in Neubauer-dishes. With these, it had shown that, e.g., plants treated with thymohydroquinone at lower humidity showed a lower degree of wilting (4) than the untreated plants. A higher wilting resistance generally is caused by an increased osmotic value of the plants which mostly is caused by an increased content of soluble sugars in the plant.

Hence the changing of the sugar metabolism by thymohydroquinone was first investigated and the effect of this substance on the activity of some enzymes taking part.

S 525

Table 2. Changing of the enzyme activities of bush beans by thymohydroquinone in water cultures (analyses of leaves).

concentrations thymohydroquinone	enzyme activities, relative			
	aldolase leaf	phosphatase leaf	saccharase leaf	amylase leaf
0	100	100	100	100
$1.5 \cdot 10^{-3}$m	(107)	96	194	108
$1.5 \cdot 10^{-5}$m	174	112	185	109

The activities of aldolase and saccharase are for instance increased in the leaves of bush beans, while those of amylase and phosphatase evidently have not changed (5). The effect of thymohydroquinone depends on concentration.

S 526

Table 3. Influence of thymohydroquinone on enzyme extracts (acetone dry powder from leaves)

concentrations thymohydroquinone	aldolase	phosphatase	saccharase	amylase
0	100	100	100	100
$1.5 \cdot 10^{-3}$m	7	109	53	106
$1.5 \cdot 10^{-4}$m	6	101	40	102
$1.5 \cdot 10^{-5}$m	7	101	66	102

If one adds thymohydroquinone directly to enzyme extracts, then the activities are more or less decreased according to concentration, though the activities in the plant were partly increased. The activity of the phosphatase is not influenced either directly or in the plant.

These results indicate that the effect of quinones or hydroquinones in the metabolism is connected with secondary processes; with this, one could think of a binding to protein or of one as glucoside.

The changing of activities of the enzymes of sugar metabolism gave the cause to investigate the content of reducing sugars in the plants.

S 527

Table 4. Influence of thymohydroquinone on the content of reducing sugars and keto acids.

Rye seedlings in Neubauer-experiment				Bean leaves in water culture (nutrient solution)
Concentrations thymohydroquinone	Reducing sugars in % of fresh weight	Reducing sugars in % of dry weight	total keto acids in % of dry weight	pyruvic acid in % of dry weight
0	0.34	3.85 ± 0.095	0.53 ± 0.031	0.21
$1.5 \cdot 10^{-3}$m	0.51	5.38 ± 0.174	0.65 ± 0.017	0.31
$1.5 \cdot 10^{-4}$m	0.40	4.32 ± 0.120	0.53 ± 0.012	-
$1.5 \cdot 10^{-5}$m	0.40	4.44 ± 0.077	0.51 ± 0.009	-

Some of the examples of experiments done with different plants and under different conditions show that with increasing concentration of thymohydroquinone not only the amount of reducing sugars but also the total keto acids (6) increased. The total keto acids were determined by the Friedemann and Haugen method (7) from the total hydrazones by extraction with acetic ester.

Pyruvic acid determined by itself, also is increased. This acid is on the one hand in correlation with sugar metabolism and on the other hand with the total acid metabolism. From this compound there results the synthesis of carbohydrates; also through further decomposition and rebuilding; there results intermediate products for synthesis of amino acids.

Thymohydroquinone showed, regarding its influence on sugar metabolism, a parallel with growth-affecting substances, e.g., 2.4-dichlorophenoxy acetic acid (8), which also increases the content of sugars in the plant.

We could show with isolated roots by means of the Warburg technique that the respiration is increased (6) in the presence of concentrations of thymohydroquinone which also stimulate the initial growth, and that those which inhibit the initial growth also cause a decrease in respiration.

Since the citric acid cycle is in connection with the respiration system of the plant, different acids (6) of this cycle were determined, to gain further knowledge about the influence of thymohydroquinone on the metabolism of plants. For this purpose, we started from the usual experimental arrangement with sand cultures in Neubauer dishes or with water cultures. 19 day old bean plants grown in water culture were put into the solutions with the substances to be examined in concentrations of 10^{-3} mol and after 3 or 4 days the leaves were analyzed. At the same time, the influence of thymohydroquinone was compared with the influence of malonic acid, of which the inhibition on succinic acid- and malic acid dehydrogenase (9) was determined by other authors.

S 528 (See S 16 in section "Introduction to the work of the Institute for Soil Biochemistry)

Thymohydroquinone increases the concentration of α-ketoglutaric acid and of citric acid evidently. The concentration of malic acid and fumaric acid is decreased.

Malonic acid (6) has the same effect with only a few exceptions. Contrary to thymohydroquinone it decreases the content of reducing sugars and of citric acid, while it increases the content of malic acid.

The mode of action of thymohydroquinone and malonic acid hence is only partly the same. From literature is known, however, that malonic acid (10) as well as quinones or hydroquinones (11) inhibit different enzymes of the citric acid cycle in high concentration.

It is almost impossible to make statements from these data about the mechanism of action of thymohydroquinone and to bring these into connection with the growth increase, since the metabolism procedures with their actions are very complicated and conclusions can not always be made from the comparison of single procedures in the metabolism on an observed total appearance. An explanation of the different results hence requires investigations on different organisms. Lower organisms are preferred since they can be handled much easier.

To get more into the changing of the metabolism by quinones, there were besides the investigations on higher plants some done on yeast (Saccharomyces cerevisiae).

The measurement of respiration in the Warburg apparatus showed by comparison of different quinones and hydroquinones a dependency of the influence from constitution and concentration, as it was also already found for the influence on the plant growth and the yield. E.g., the oxygen consumption (12) at a 10^{-5} molar concentration of thymohydroquinone was increased.

The determination of the respiration quotient shows, with concentration of 10^{-3} to 10^{-4}m of some of the quinones used, a shifting of the metabolism toward anaerobiosi (i.e., they stimulate the fermentation) while with other quinones no influence can be found.

During the investigations, the glucose decomposition (12) was measured in presence of thymohydroquinone by its decrease in the exterior solution which only was increased with concentrations increasing oxygen. The results about the increased glucose decomposition with concentrations increasing the growth agree well with the increased oxygen consumption in Warburg experiments.

The concentration of pyruvic acid is only increased in case of respiration stimulating concentrations in the first hours, while later on in the experiment it is similar to that of the control by consumption of glucose.

The content of total carbonyl compounds is with same concentration almost unchanged.

S 529

Table 6. Glucose consumption, concentration of ketonic acids and total carbonyl-compounds in yeast under influence of thymohydroquinone with 1% glucose in the exterior solution.

Thymohydro-chinon	1h	2h	3h	4h	5h	6h
			zeit			
			Glucoseverbrauch in mg %			
Kontrolle	260	480	620	710	825	930
10^{-5}m	280	520	655	735	820	920
			Gehalt an Ketosauren in mg % Brenztraubensaure			
Kontrolle	5.6	8.0	9.4	12.7	13.2	13.7
10^{-5}m	6.6	9.1	9.9	13.0	13.2	13.9
			Gehalt an Gesamtcarb. verb. in mg % Brenztraubensaure			
Kontrolle	10.3	11.5	14.6	16.9	19.8	19.6
10^{-5}m	9.4	12.3	14.0	16.1	20.2	20.2

S 530

Table 7. Glucose consumption and acetaldehyde and alcohol formation in mg after 60' test.

thymo-hydroquinone	mg. Glucose-verbrauch	mg Aethyl-alkohol	mg. Acetaldehyd
Kontrolle	405	170	20
10^{-3}m	150	25	53
10^{-4}m	318	88	40
10^{-5}m	495	198	24

With anaerobic conditions under nitrogen, thymohydroquinone at a concentration of 10^{-5} resulted in a decreasing of glucose in the exterior solution and an increased formation of ethyl alcohol. In higher concentrations, the glucose consumption was decreased and the acetaldehyde concentration was increased. This result is due to the inhibition of the alcohol dehydrogenase. In connection with the preceding experiment, it can be said that the alcohol dehydrogenase is more sensitive against the influence of quinones and hydroquinones than the pyruvic acid decarboxylase, since the content of pyruvic acid is only increased slightly.

Furthermore, the influence of thymohydroquinone on the phosphorylation (13) was investigated.

S 531

Table 8. Decreasing of the inorganic P under aerobic and anaerobic conditions in 1% glucose solution.

		mg P - Verbrauch/g Hefe aerobic n. 10 min.	mg P - Verbrauch/g Hefe anaerobic n. 20 min.
Kontrolle		1.28	1.15
Thymohydrochinon	10^{-3}m	0.90	0.85
	10^{-4}m	1.03	1.02
	10^{-5}m	1.12	1.01
2,4-DNP	10^{-3}m	0.65	0.69
	10^{-4}m	0.85	0.80

In any case, the phosphorylation is decreased by addition of thymohydroquinone. The concentration of 10^{-5} thymohydroquinone increased - as mentioned already - the oxygen consumption. The values for phosphorylation indicate that thymohydroquinone in this concentration has a slight uncoupling effect. For comparison, experiments with 2,4-dinitrophenol (2,4-DNP) were done in concentrations which increase oxygen consumption (14). From the data it can be seen how much stronger 2,4-DNP uncouples.

While 2,4-DNP in concentrations of 10^{-3} to 10^{-4} does not increase plant growth and thymohydroquinone in a concentration of 10^{-5} increases plant growth, a slight uncoupling effect seems to be important for plant growth.

The Pasteur effect is brought into connection with the phosphorylation processes (15). Therefore, we have investigated the influence of thymohydroquinone on this effect. As measurement for the influence of a substance on the Pasteur effect, the Pasteur quotient is the ratio of aerobically consumed glucose to anaerobically consumed glucose.

S 532 (See next page)

From the table can be seen that the Pasteur quotient is changed differently by the different high concentrations of thymohydroquinone. With a concentration of 10^{-5}m, the Pasteur quotient is with increased aerobic and anaerobic glucose consumption below that of the contol; this result is due to stronger increasing of the glucose consumption with thymohydroquinone than with 2,4-DNP.

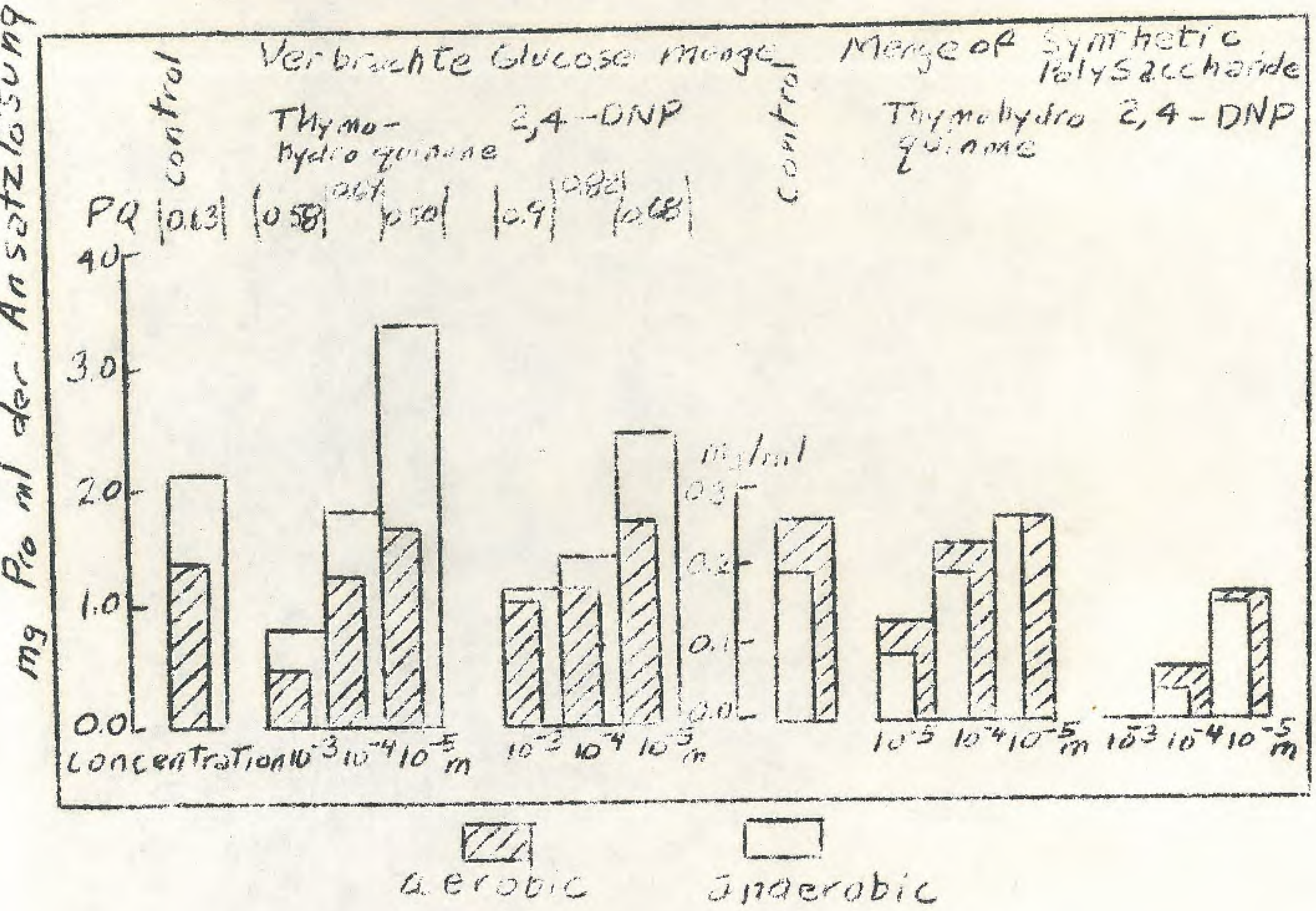
Besides, in contrast to 2,4-DNP, in the tests with thymohydroquinone the polysaccharide synthesis is less inhibited.'

The experiments about the influence of quinones or hydroquinones on the metabolism of plants can be summarized as follows:

In concentrations between 10^{-4} and 10^{-6} mol/L with low relative humidity, an increasing of the yield is found. This is due to changing of the metabolism, of which so far the sugar- and acid-metabolism has been investigated thoroughly. In the further course of the investigations, an increasing of respiration of lower organisms, a slight uncoupling of the phosphorylation, and an influence on the Pasteur quotient was found.

S 532

Table 9. Influence of thymohydroquinone and 2,4-DNP on aerobically and anaerobically consumed glucose amount in one hour as well as on the polysaccharides synthesized during this time.



The influence of thymohydroquinone and 2,4-DNP auf die aerobic and anaerobic in einer stunde Verbrauchte Glucose menge sowie auf die in dieser. Zeit synthetisierten Polysaccharide.

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