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Roach, W. A. 1930. Sulphur as a soil fungicide against the potato wart disease organism. *The Journal of Agricultural Science*. 20 (1), pp. 74-96.

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SULPHUR AS A SOIL FUNGICIDE AGAINST THE POTATO WART DISEASE ORGANISM.

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(With Eight Text-figures.)

INTRODUCTION.

THE variability in the toxicity of sulphur, when used as a fungicide against the organism that causes the Wart Disease of Potatoes, has formed the subject of a number of earlier papers (2, 10, 11, 12) and led in 1926 to an investigation in the laboratory into the underlying causes. The acidity that results from the oxidation of the sulphur to sulphuric acid, a process which is known to complete itself in soil in a few weeks, having been found to be insufficient to account for the fungicidal action (2, 10, 12), the idea suggested itself that some other compound, formed as a result of the interaction of sulphur and soil, might be responsible. In assessing the importance of any such compound, account has to be taken both of its concentration and of its degree of toxicity. At the outset, therefore, the investigation proceeded along two lines: (1) the exploration of the chemical changes undergone by sulphur in soil, and (2) the determination of the toxicities of compounds formed or at all likely to be formed under these conditions. The second part of the problem has already been the subject of a communication (12).

The first-named part of the investigation, viz. the exploration of the chemical changes undergone by sulphur in soil, which forms the subject of this paper, proved much more difficult. Experiments of as simple a nature as possible were carried out to determine what kinds of compounds are formed when sulphur is incorporated with soil, and to get some idea of the conditions favouring the formation of any such compounds as happened also to be toxic to the Wart Disease fungus. These qualitative, or only partially quantitative, experiments were to have been preliminary to more carefully planned quantitative ones, but owing to the writer's transference to another sphere of work even the

¹ The work described on pp. 85-92 was carried out after the writer had taken up his new appointment.

preliminary experiments had to be somewhat curtailed. Nevertheless the results obtained, so far as they go, do seem to throw some light on the problem and do at least suggest a possible mode of action of sulphur as a soil fungicide, hence they are put on record here. Compounds which it seems at all likely would be formed as a result of the interaction between sulphur and soil fall conveniently into four main classes:

- (i) Sulphuretted hydrogen and other sulphides.
- (ii) Compounds intermediate in state of oxidation between sulphur and sulphuric acid.
- (iii) Sulphuric acid and sulphates.
- (iv) The persulphuric acids and their salts, together with hydrogen peroxide and ozone.

These four classes are readily distinguished chemically; sulphuric acid and sulphates alone give an immediate precipitate with barium chloride, whereas members of the first two classes give such a precipitate only after preliminary treatment with strong oxidising agents; members of the fourth class alone give a blue coloration with acidified potassium iodide and starch. The first step, therefore, was to seek evidence of the formation of any members of these classes of compounds under conditions as similar as possible to those in the field. The tests were carried out mainly on aqueous extracts of the soil.

EXPERIMENTAL METHOD.

Soil. Most of the experiments were carried out mainly with two kinds of soil:

1. *Ormskirk* soil, taken from the field at the Potato Testing Station of the National Institute of Agricultural Botany. This was a typical black, peaty, sandy potato soil.

2. *Rothamsted* soil, taken from the electro-culture enclosure. This soil was a stiff clay, containing numerous small pieces of calcium carbonate.

The soil in each case was allowed to dry under cover until it could readily be crumbled, it was then rubbed through a 2 mm. sieve. The portion passing through was thoroughly mixed and moistened with water by means of a spray until a handful on being compressed stuck together, but on gentle rubbing with the finger disintegrated again into its original particles. Plants grow readily in such a soil. After thorough mixing a quantity was weighed out and spread in a uniform layer of about $\frac{1}{2}$ -inch thickness on a clean sheet of paper. The requisite quantity of finely ground and sifted sulphur was then distributed as uniformly

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as possible through a "100 mesh" sieve over the soil. After a preliminary mixing by hand the soil-sulphur mixture was transferred through a 2 mm. sieve to another piece of paper, care being taken that each charge of the sieve consisted of portions from all parts of the heap. This sifting and mixing process was repeated at least six times.

The prepared soil-sulphur mixture was usually weighed out in 100 gm. lots and put into glass test-tubes placed in constant temperature baths at 0°, 15° and 30° C. respectively, the ends of the tubes being either open to the air or lightly plugged with cotton wool. The moisture content was made up periodically by dropping water slowly from a pipette on to the mixture, care being taken not to cause the particles to run together.

Methods of soil extraction. Three methods of extraction of the soil-sulphur mixture were used.

1. The 100 gm. sample was placed in a glass tube provided at the lower end with a rubber stopper through which passed a No. L. 1 Chamberland filter candle. Water was added, the upper end of the tube then closed with a rubber stopper and the whole thoroughly shaken by hand. The candle was connected to a filter pump through a flask, and the upper rubber stopper removed. This method gave a small quantity of extract within a few minutes of the wetting of the mixture, and it was convenient for use when speed was essential. It was, however, inconvenient for quantitative work, because the rate of filtration slowed down as the pores of the candle became clogged, and unless some salt was added with the second lot of extracting water the clay became deflocculated and rendered a second filtration very slow indeed. (See Fig. 1.)

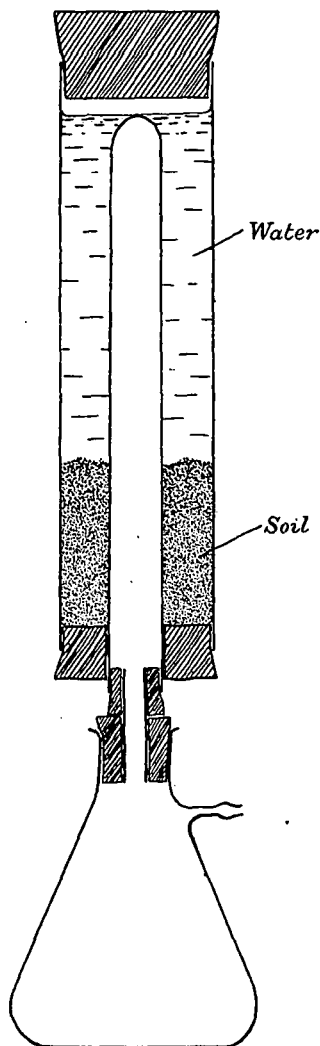


Fig. 1. Apparatus No. 1 for obtaining rapidly a small quantity of extract from soil.

2 (a). The 100 gm. sample of soil-sulphur mixture was placed in a Soxhlet extraction thimble standing in a glass funnel, and water was allowed to drop on it at such a rate that it moved from crumb to crumb by capillarity without the intervening air-spaces becoming water-logged. In this way the most concentrated soil extract was obtained, and it was perfectly clear. 5 c.c. of normal sulphuric acid added to and mixed with 100 gm. of the soil-sulphur mixture could be extracted completely in about 12 hours, as may be seen from the following figures which are represented graphically in Fig. 2.

	Volume in ml.	\equiv ml. <i>N</i> /10 NaOH	Total sulphur extracted by 1 ml. water %
1st percolate	50	13.3	0.519
2nd "	70	24.3	0.678
3rd "	50	12.2	0.471
4th "	60	1.4	0.046
5th "	50	0.0	0.00
		51.2	

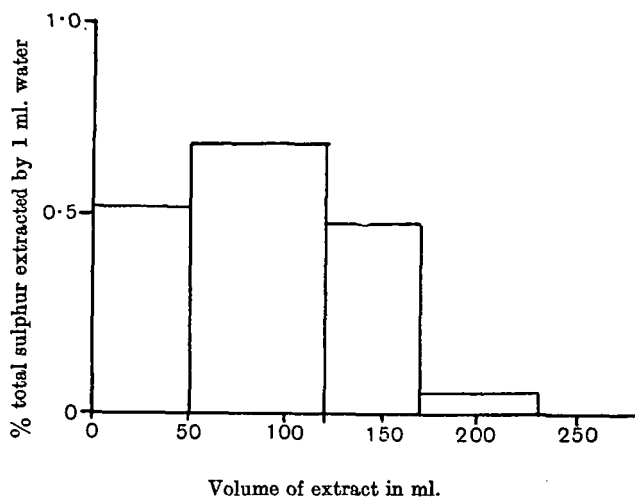


Fig. 2. Amounts of sulphur extracted per ml. water by successive portions of water.

Sulphuric acid added \equiv 51.25 ml. *N*/10 NaOH. Hence 230 ml. water extracted all the sulphuric acid from 100 gm. soil.

2 (b). For extracting larger quantities use was made of an elongated Büchner funnel, the elongation consisting of a glass tube of the same diameter as the funnel and fixed thereto by means of surgical rubber tape.

3. The apparatus shown in Fig. 4 was used to obtain extracts under rather less aërobic conditions than in the last method.

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Estimation of sulphur in soil.

Extraction. Acetone was found unsatisfactory as a solvent, because it extracted from soil a dark gummy substance which, on subsequent

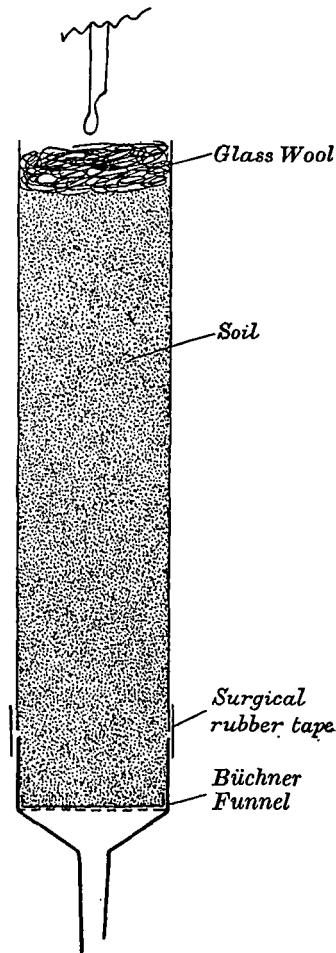


Fig. 3. Apparatus No. 2 b for extracting larger quantities of soil.

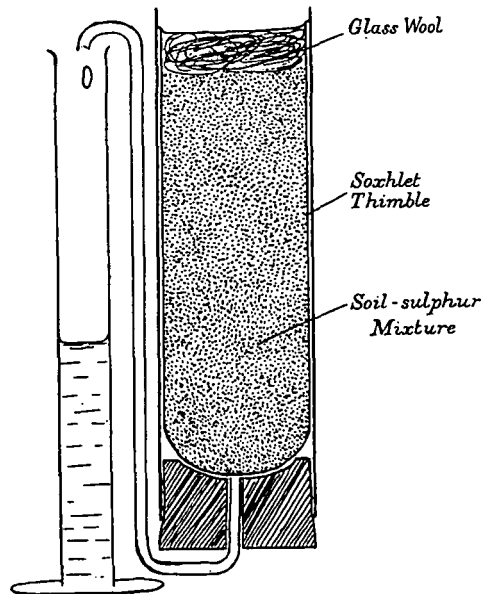


Fig. 4. Apparatus No. 3 for obtaining soil extract under partially aerobic conditions.

oxidation of the extracted sulphur with bromine and nitric acid or by other means, gave a yellowish resinous substance highly resistant to oxidation. Carbon tetrachloride, however, extracted the sulphur almost free from the contaminating gummy substance, even from Ormskirk soil, which was particularly troublesome when acetone was used.

Estimation. The carbon tetrachloride extract was evaporated to

dryness on the water bath. The neck of the flask was closed with a ground-in tap funnel (the joints were ground so that the flasks would fit either the Soxhlet extractors or the ground-in tap funnel), Fig. 5. The flask was evacuated and the tap closed. For 0.1 gm. sulphur 10 c.c. *N/1* caustic soda was added through the tap without admitting any air and the flask was replaced on the water bath and left there until all the sulphur had dissolved. In this way any loss of volatile sulphur compounds was avoided. The oxidation was carried out by means of hydrogen peroxide, or, more readily, by potassium bromate and hydrochloric acid, as in the method of Treadwell and Mayr⁽¹³⁾. The sulphate formed was estimated by one of the recognised methods.

Absence of sulphuretted hydrogen and sulphides.

In all the experiments described tests were made for sulphuretted hydrogen and sulphides in three ways: (a) by hanging a lead acetate paper just above the soil-sulphur mixture, (b) by moistening such a paper with the extract, and (c) by placing a little soil-sulphur mixture on the paper and carefully dropping enough water on the soil to cause it to adhere to the paper. The paper invariably remained white; and numerous other experiments not referred to also gave the same negative results. The temperature varied from 0° to 30° C.; the soil varied from the typical Rothamsted field soil through a rich allotment soil from Rothamsted to the peaty sandy Ormskirk soil, and aëration varied down to complete water-logging for more than a week on end. It seems unlikely, therefore, that sulphuretted hydrogen or other sulphides are ever formed under field conditions in sufficient quantity to have any appreciable toxic action on the Wart Disease fungus, especially in view of their low degrees of toxicity.

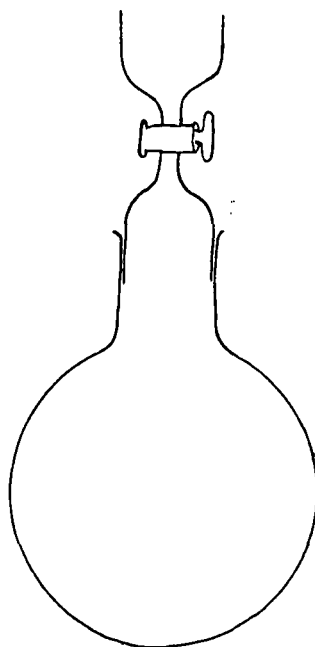


Fig. 5. Oxidation flask for method of Treadwell and Mayr.

Absence of persulphates, etc.

Numerous tests made on soil-sulphur mixtures kept under even more widely varying conditions gave no clear indications of the formation of

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any substance able to liberate iodine from slightly acidified potassium iodide; whenever the blue starch-iodide colour was given in a paper test over sulphur-treated soil or in air aspirated through such soil a similar colour was also given when untreated soil was substituted for the sulphur-treated soil. In no single experiment carried out at temperatures likely to be met with in the soil (say up to 30° C.) was there any difference in the behaviour of untreated and sulphur-treated soils as judged by the intensity of the blue colour developed. In spite of the high toxicity of persulphuric acid it seems unlikely that enough could have been formed under these conditions to explain the toxicity of the sulphur. In experiments carried out entirely in solution on soil extracts the results were more definitely negative. A blue colour was never obtained on acidifying and adding potassium iodide and starch solution to a colourless soil extract.

Compounds of classes (i) and (iii) therefore, are not further dealt with in this communication.

Evidence for the formation of compounds intermediate in state of oxidation between sulphur and sulphuric acid.

The following compounds, among others, belong to this class: thio-sulphuric acid, $\text{H}_2\text{S}_2\text{O}_3$; pentathionic acid, $\text{H}_2\text{S}_5\text{O}_6$; tetrathionic acid, $\text{H}_2\text{S}_4\text{O}_6$; trithionic acid, $\text{H}_2\text{S}_3\text{O}_6$; dithionic acid, $\text{H}_2\text{S}_2\text{O}_6$; sulphurous acid, H_2SO_3 ; and the salts of these acids.

The experimental procedure in testing for the accumulation of these substances in soil-sulphur mixtures was to treat the soil extract with excess of barium chloride, spin down the barium sulphate precipitate on the centrifuge, then treat the solution with potassium chlorate and hydrochloric acid, when a further precipitate would indicate the presence of one or more of these compounds. (It has already been stated that tests for sulphides were uniformly negative.) A number of preliminary experiments of this kind indicated that in the Rothamsted soil to which sulphur had been added, appreciable quantities of the sulphur were oxidised in the course of a few days to compounds which, on oxidation with chlorine or other strong oxidising agents, gave sulphate. On a number of occasions tests were made for thiosulphate and sulphite by precipitating the sulphate with excess of barium chloride, removing the precipitate and titrating with iodine. In all experiments except one 2 drops of decinormal iodine solution were sufficient to give the "starch-iodide" blue; in the one experiment, as will be seen later (p. 82), 0.2 c.c. was necessary. This evidence, though perhaps suggestive,

is insufficient to establish the presence of thiosulphate or sulphite. The fact that the addition and slight combination of iodine was never followed by a further precipitate of barium sulphate proved that if either of these two compounds was present it was thiosulphate, which, as is well known, gives tetrathionate with iodine, whereas sulphite forms sulphate. Whenever an appreciable further precipitate was formed after treatment with a strong oxidising agent, another portion of the solution always gave a white, or sometimes even a yellow, precipitate of sulphur, on the addition of 20 per cent. caustic soda. The solution also gave the other tests characteristic of polythionates. Hence the solutions definitely contained pentathionate and possibly other polythionates, though the lower polythionates cannot be detected by any known qualitative test in the presence of pentathionate. The characterisation of the polythionates in the presence of each other can be done only by quantitative means.

The few results of some work along quantitative lines did not justify the drawing of definite conclusions.

A few of the experiments proving the presence of polythionates in sulphur-treated soil are given in more detail below. In most of these the amount of sulphur added was chosen to correspond with a dressing of 1 ton per acre in the field, *i.e.* 0.1 gm. sulphur was added to 100 gm. of the damp soil.

EXPERIMENTAL RESULTS.

I. *Experiment begun, 28. viii. 26.*

Rothamsted soil, 5000 gm., mixed with 5 gm. sulphur (as used at Ormskirk in 1925). Temperature, 30° C.

Untreated soil. An extract of 100 gm. of soil was made by means of apparatus 1. The filtrate was perfectly clear and neutral in reaction. It gave only a barely perceptible turbidity with barium chloride and with benzidine; hence it was practically free from sulphate. After oxidation with hydrogen peroxide or potassium chlorate and hydrochloric acid and subsequent addition of barium chloride, or benzidine, the solution became only very faintly turbid; hence it was free from all but minute traces of all sulphur compounds.

Soil and sulphur mixture. The same results as above were obtained with the aqueous extract made from the sulphur-treated soil immediately after the sulphur was added; hence there was no appreciable immediate reaction between the soil and sulphur.

7. ix. 26 (10 days after beginning of experiment).

Tube No. 37 percolated by method 2 and sulphate determined by Raschig's benzidine method.

1st 80 c.c. of percolate	≡ 5.49 c.c. <i>N</i> /10 NaOH
Next 80 c.c. of percolate	≡ 0.00 „ „
	5.49 „ „
	≡ 0.018 gm. sulphur as sulphate,
	<i>i.e.</i> 18 per cent. sulphur oxidised to sulphate.

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8. ix. 26. Test for forms of sulphur other than sulphur and sulphate.

Two tubes of soil were percolated by method 2, till the percolate no longer gave a precipitate with barium chloride. Each percolate was treated with excess of barium chloride and the precipitated barium sulphate centrifuged off, washed and weighed.

Weights of barium sulphate: (1) 0.0988 gm., (2) 0.1069 gm. The centrifugate, which was perfectly clear, was boiled with hydrogen peroxide. There was a further small precipitate of barium sulphate, indicating that the solution contained some oxidisable sulphur compound soluble in water.

Weights of barium sulphate: (1) 0.0458 gm., (2) 0.0467 gm. Hence in 11 days at 30° C. about

14 per cent. of the sulphur was in the form of sulphate,

6 per cent. of the sulphur was present as some compound oxidisable by hydrogen peroxide to sulphate,

and the remaining

80 per cent. presumably unchanged.

II. Experiment begun, 21. i. 27.

Rothamsted soil, 0.2 per cent. sulphur. Temperature, 30° C.

2. ii. 27 (12 days after beginning of the experiment).

100 gm. of soil percolated by method 2 and oxidisable sulphur estimated by the method of Treadwell and Mayr.

1st percolate	50 c.c. (12.30–6 p.m. = 5½ hr.)	≡ 7.00 c.c. $N/10$ $Na_2S_2O_3$.
2nd	40 ,, (during night)	≡ 2.55 ,, ,,
3rd	50 ,, (10 a.m.–12.30 p.m. = 2½ hr.)	≡ 1.35 ,, ,,
4th	50 ,, (12.30–5.30 p.m. = 5 hr.)	≡ 1.02 ,, ,,
5th	50 ,, (during night)	≡ 0.22 ,, ,,
		12.15 ,, ,,

12.15 c.c. 0.998 $N/10$ $Na_2S_2O_3$ ≡ 0.0097 gm. S_5O_6 sulphur (or about 0.5 per cent.),
or 0.015 gm. S_5O_6 sulphur (0.7 per cent.).

8. ii. 27 (20 days after beginning of experiment).

Extracts of two separate tubes made by method 3.

No. 1. 1st 150 c.c. extract treated by Treadwell and Mayr method

≡ 7.3 c.c. 0.998 $N/10$ $Na_2S_2O_3$,

or 0.0058 gm. S_5O_6 sulphur, or about 2.9 per cent.

No. 2. 1st 150 c.c. extract decolorised 1 drop but not 2 drops $N/10$ iodine.

Hence no appreciable quantity of S_2O_3 or of SO_3 was present.

Two tubes extracted in the same way as the above two but more slowly; the extracts being united, mixed and divided into two equal parts.

1st half decolorised 1 drop but not 2 drops of $N/10$ iodine.

2nd half when oxidised in the Treadwell and Mayr apparatus

≡ 9.1 c.c. $N/10$ $Na_2S_2O_3$,

≡ 0.0073 gm. S_5O_6 sulphur, or 3.6 per cent.

9. ii. 27 (21 days after commencement of the experiment).

Two lots of 500 gm. sulphur-treated soil extracted by method 2 a.

Polythionate sulphur:

(1) \equiv 75.1 c.c. *N*/10 bromine or 0.06 gm. S_5O_6 sulphur (6 per cent.);

(2) \equiv 50.0 " " " 0.04 " " (4 per cent.).

(1) 50 c.c. solution \equiv 0.20 c.c. *N*/10 iodine solution;

(2) " " \equiv 0.20 " "

This was the only indication of any appreciable quantity of thio-sulphate or sulphite being formed. This evidence is insufficient on which to base the conclusion that a definite amount of thiosulphate or sulphite was present in the extract.

There is definite evidence therefore that in Rothamsted soil kept at 30° C. pentathionate (accompanied possibly by other polythionates) is formed in quantities up to 6 per cent. of the sulphur added. No evidence was obtained in these experiments of the formation of any appreciable quantities of sulphur compounds intermediate in state of oxidation between sulphur and sulphuric acid in Rothamsted soil kept at 15° and 0° C. respectively.

Ormskirk soil. Similar but less extensive experiments were carried out on Ormskirk soil; in none of the experiments was there any evidence of the formation of appreciable quantities of such compounds.

Hence we have definite evidence of only one compound being formed in any appreciable quantity as an intermediate product in the oxidation of sulphur to sulphuric acid, viz. pentathionate; but possibly this was accompanied by other polythionates. None of the polythionates shows any toxicity towards the Wart Disease fungus, however, hence their formation on sulphur-treated soil throws no direct light on the mode of fungicidal action of sulphur; but it does give a hint of a conceivable mode of action. It is well known that polythionates are formed when a thiosulphate is acidified, hence the question arises: was the pentathionate found in the soil solution formed from pre-existing thiosulphuric acid? *i.e.* does the oxidation take place in some such steps as the following:

sulphur \rightarrow thiosulphuric acid \rightarrow polythionic acids \rightarrow sulphuric acid.

Much support may be found for this view in the work on the oxidation of sulphur in soil and other habitats and by micro-organisms in culture fluids reviewed by Joffe(8) and Guittonneau and Keiling(6). Probably all of these changes are brought about mainly by micro-organisms, but chemical evidence, as reviewed by Bassett and Durrant(1), also supports the view that some such series of changes as the above takes place.

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The fact that the presence of thiosulphate in appreciable quantities was not definitely established by the foregoing experiments is not evidence against the above view. The amount accumulating in the soil solution will be conditioned both by the rate of its formation from sulphur and by the rate of its change into pentathionate. If, now, the soil be extracted, the sulphur is removed with the soil from the reaction solution and the source of the thiosulphate disappears; but there is no reason for supposing that the change of thiosulphate into pentathionate is interfered with materially; thus there is reason for supposing that the amount of thiosulphate in the solution will decrease immediately the sulphur is separated from it. Hence to test for its formation with any degree of certainty it would be necessary either (a) to remove the thiosulphate as an insoluble compound actually from the soil solution itself, (b) to convert it into a compound not changed to pentathionate, or (c) to arrest all these changes suddenly such as by very low temperature and subsequent extraction with alcohol. An opportunity to carry out such experiments did not present itself; but recently work has been done which has shown that thiosulphuric acid can exist in a free state; a comparison has been made between the degree of toxicity of certain solutions and their content of thiosulphuric acid. This helps to assess the value of the suggestion that sulphur acts as a soil fungicide through the formation of thiosulphuric acid.

The existence in a free state and the degree of stability of thiosulphuric acid.

The existence of thiosulphuric acid in a free state is commonly denied in text books, thus Ephraim⁽³⁾ states: "the free acid has not been isolated, as it decomposes at once into sulphurous acid and sulphur," but, as already indicated in a former paper ⁽¹²⁾, p. 181, even after 12 hours an S/1000 acidified thiosulphate solution remained perfectly clear. Even much stronger solutions remain clear for shorter lengths of time. Though acidified thiosulphate decomposes in other ways giving rise to products other than sulphur and sulphur dioxide⁽¹⁾, these facts do rather suggest that the decomposition of free thiosulphuric acid is not instantaneous but requires a definite period of time for completion and that the final equilibrium solution in each case will contain definite amounts of free thiosulphuric acid. Before this idea could be tested analytical methods have had to be modified for the purpose.

The readiest method of estimating thiosulphate is by titration with standard iodine solution, but this method is not applicable unmodified in the presence of sulphite, since sulphite also is oxidised by iodine.

The sulphite may be precipitated and removed, or more simply it may be locked up by treatment with formaldehyde in a compound in which it is not acted upon by iodine; the thiosulphate may then be titrated with iodine (assuming sulphides to be absent, as in fact they were in all the solutions examined). The necessary conditions for concentrated solutions were worked out by Kurtenacker(9). Certain refinements, however, are necessary before the method is applicable to dilute solutions. The modified method will be described before giving the results obtained by it.

Estimation of thiosulphate in presence of sulphite by a modification of Kurtenacker's method.

The essence of Kurtenacker's method is to neutralise the solution to phenol phthalein with ammonia to remove any strong acids, add an excess of formaldehyde, then render acid with acetic acid and titrate immediately after acidification with iodine solution. Under these conditions the sulphite is rendered almost completely inactive towards iodine, the excess formaldehyde has no measurable effect on iodine and any formaldehyde thiosulphuric acid formed is sufficiently unstable to allow of rapid titration of the thiosulphate part of it.

The method exactly as recommended by Kurtenacker was found satisfactory for the relatively concentrated solutions with which he worked, but in more dilute solutions it became inapplicable. The following modifications were found necessary or considered desirable.

Excess of potassium iodide. For iodine-thiosulphate titrations in $N/10$ solution the amount of the excess of potassium iodide added does not matter within limits, though even in such concentrated solutions a large excess causes the colour with starch to be brown rather than blue and to fade rather rapidly making the end-point somewhat indefinite. No experimental evidence could be found for the desirability of the addition of any potassium iodide besides that usually contained in the iodine solution. As will be seen later, the error, through omitting to add further potassium iodide, is only a small fraction of a drop of $N/10$ iodine solution. The influence of the concentration of potassium iodide in the solution titrated is well seen by determining the amount of standard iodine solution necessary to give a definite colour in presence of varying amounts of potassium iodide.

Each solution contained 50 ml. water, 1 ml. 0.5 per cent. starch, 1 ml. glacial acetic acid.

ml. 10 % KI added	0	1	5	10
1 drop $N/100$ iodine	No colour	Pure blue	Not pure blue	Reddish blue
2 drops ,,	Faint pure blue	—	—	—
3 drops ,,	Definite pure blue	—	—	—

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Thus 2 drops of $N/100$ iodine are sufficient to give a definite though faint coloration in 50 ml. solution and 3 drops an easily recognised blue colour, without the addition of any potassium iodide other than that in the iodine solution. The addition of 1 ml. 10 per cent. potassium iodide to 50 ml. solution (*i.e.* 0.2 per cent. KI) was sufficient to cause 1 drop of $N/100$ iodine solution to give a definite pure blue colour. The colour became more and more reddish as more potassium iodide was added, becoming brown and finally practically disappearing when large excesses were added.

With $N/1000$ iodine using the same solution as the above the following results were obtained:

ml. 10 % KI added	0	0.25	0.5	1
1 drop $N/1000$ iodine	Colourless	Colourless	Colourless	Colourless
5 drops "	"	Light blue	Light blue	Light blue
10 drops "	"	Blue*	Blue*	Blue*
25 drops "	Just perceptible colour	—	—	—
30 drops "	Blue*	—	—	—

Solutions marked (*) were of approximately the same colour. On the further addition of 5 ml. 10 per cent. KI the blue colour became tinged with red but was hardly any deeper.

Hence the presence of 0.2 per cent. potassium iodide was sufficient to cause 1 drop of $N/100$ iodine, to give an easily distinguishable blue colour and 5 drops of $N/1000$ iodine to give a definite light blue colour. This concentration was accepted as the best for the present purpose.

Excess of formaldehyde. Experiments were carried out to determine the minimum amounts of formaldehyde necessary to render inactive towards iodine the various amounts of sulphite likely to be dealt with. Thus using 1 ml. 35 per cent. formaldehyde in 50 ml. $S/200$ sodium sulphite (+ 1 ml. glacial acetic acid, 1 ml. 10 per cent. KI, 1 ml. 0.5 per cent. starch), 1 drop of $N/100$ iodine gave a definite colour. With quantities of formaldehyde less than 1 ml. more than 1 drop of $N/100$ iodine was necessary.

In order to determine whether this amount of formaldehyde had any serious effect on the iodine titration under the experimental conditions the following solutions were made up:

A	B
50 ml. water	50 ml. water
1 ml. 35 % formaldehyde	
1 ml. 10 % KI	1 ml. 10 % KI
1 ml. glacial acetic acid	1 ml. glacial acetic acid
1 drop $N/100$ iodine	1 drop $N/100$ iodine
1 ml. 0.5 % starch solution	1 ml. 0.5 % starch solution

The colour of solution *A* was visible even without using a beaker of pure water for comparison; hence the difference due to an excess of 1 ml. 35 per cent. formaldehyde was less than 1 drop of *N*/100 iodine.

The colour of solution *A*, however, was fainter than that of solution *B*; hence even this small amount of formaldehyde had some small effect on the iodine, so it was considered undesirable to increase the amount more than necessary. This amount was found satisfactory for *S*/20, *S*/100 and *S*/1000 solutions.

The modified method, therefore, is as follows: to 25 or 50 ml. solution are added (in the following order) enough ammonia to render neutral to phenol phthalein, 1 ml. 35 per cent. formaldehyde, 1 ml. 10 per cent. potassium iodide and 1 ml. glacial acetic acid; immediately after the addition of the acetic acid the solution is titrated with iodine.

The following results demonstrate the degree of accuracy readily attainable:

1. 25 ml. *S*/100 $\text{Na}_2\text{S}_2\text{O}_3$ + 25 ml. water + 1 ml. glacial acetic acid + 1 ml. 10 per cent. potassium iodide + 1 ml. 0.5 per cent. starch
 \equiv (12.45), (12.44), 12.49, 12.49 \equiv 12.49 ml. *N*/100 iodine solution.
2. The above solution + 1 ml. 35 per cent. formaldehyde
 \equiv 12.49 ml. *N*/100 iodine solution.
3. Solution 2 with water replaced by 25 ml. *S*/100 Na_2SO_3
 \equiv 12.49, 12.51 \equiv 12.50 ml. *N*/100 iodine solution.

By the time the above analytical method had been worked out it was no longer possible to test the toxicities of solutions simultaneously with their analysis. The best that could be done therefore was to analyse solutions made up to be as similar as possible to certain of those the toxicities of which had already been determined (12). In that paper (p. 181) are given the toxicity figures for a double series of solutions of thiosulphuric acid in the presence of excess of sulphuric acid in the one series and of sulphurous acid in the other series.

When a thiosulphate is acidified a variety of compounds are formed which exist more or less in equilibrium with each other. As is well known, if to such a decomposing compound one of its products of decomposition is added, the decomposition tends to be arrested and so the equilibrium mixture tends to contain more of the undecomposed compound. Thus sulphurous acid being known to be a decomposition product of thiosulphuric acid it might be expected that the substitution of sulphurous for sulphuric acid in the solution of thiosulphuric acid

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would result in changes in the equilibrium solution obtained. In particular the relation between any undecomposed thiosulphuric acid and its decomposition products would be upset. That some such modification of the equilibrium mixture was brought about is shown by the fact that

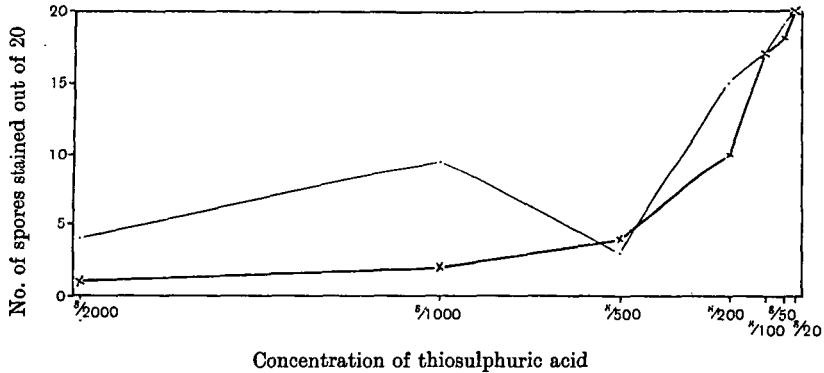


Fig. 6.

Connection between concentration and toxicity of thiosulphuric acid solutions in presence of excess of sulphuric acid — • — •, sulphurous acid — x — x. Spores treated for 24 hours. Results taken from Roach and Glynne (12).

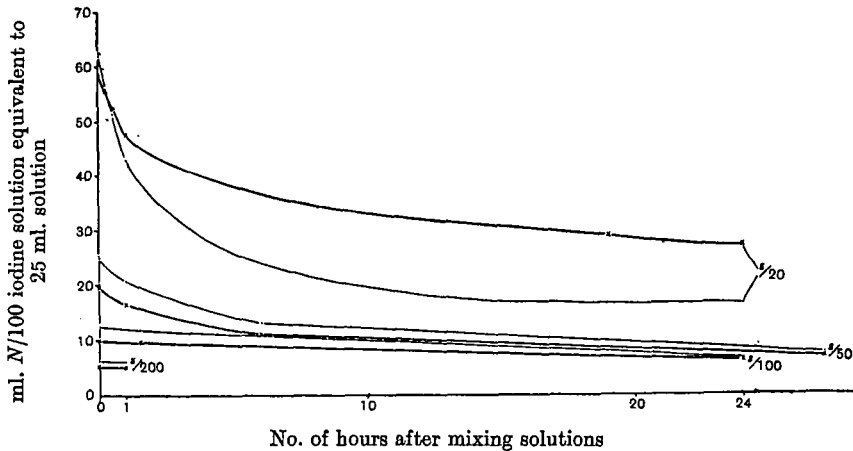


Fig. 7.

Concentration of thiosulphuric acid after varying lengths of time in presence of excess of sulphuric acid — • — •, sulphurous acid — x — x.

the separation of sulphur was different in the two series of solutions, hence a comparison of the toxicities of such solutions with their thiosulphuric acid contents might be expected to throw light on the question of the degree of connection between them. As may be seen from Fig. 6 toxicities of corresponding solutions of the two series are the same

within the admittedly large experimental error. Similar solutions were made up and their thiosulphuric acid contents determined after standing for varying lengths of time. The analytical results are shown in Table I and Fig. 7. The figures given are the numbers of ml. *N*/100 iodine solution equivalent to 25 ml. of the solution tested. As a check an aliquot portion of each solution was titrated before the acid was added and in addition some of the solutions were neutralised and titrated immediately after the acid was mixed. It will be seen from Table I that there is no justification for the general belief that thiosulphuric acid is completely decomposed as soon as liberated; for at the end of the few minutes necessary to mix the acid and thiosulphate solutions, pipette off the aliquot portion and neutralise it, less than 1 per cent. of the thiosulphate had disappeared.

Table I. $\text{H}_2\text{S}_2\text{O}_3$.

	<i>S</i> /20		<i>S</i> /50		<i>S</i> /100		<i>S</i> /200	
	Excess H_2SO_4	Excess H_2SO_3	Excess H_2SO_4	Excess H_2SO_3	Excess H_2SO_4	Excess H_2SO_3	Excess H_2SO_4	Excess H_2SO_3
Before mixing	62.4	58.6	25.25	19.2	12.65	9.96	6.265	5.2
Immediately after mixing	61.94	58.4	25.05	—	12.6	—	—	—
$\frac{1}{4}$ hr. after mixing	56.0	55.65	—	—	—	—	—	—
$\frac{1}{2}$ hr. "	51.1	52.2	—	—	—	—	—	—
1 hr. "	43.0	47.4	20.01	16.5	—	—	6.21	5.15
$1\frac{1}{2}$ hr. "	—	—	—	—	11.9	9.95	—	—
19 hr. "	—	29.0	13.0	11.0	—	—	—	—
24 hr. "	16.5	27.25	—	—	6.9	6.3	—	—
27 hr. "	—	—	7.65	7.0	—	—	—	—
44 hr. "	10.0	—	—	—	—	—	—	—
7 days after mixing	—	—	3.35	2.35	2.7	2.35	2.83	2.8

Next, the increase of stability of the thiosulphuric acid with dilution will be noticed; thus the titration figure for the *S*/20 thiosulphuric acid with excess of sulphuric acid in 24 hours decreased from 62.4 to 16.5 (*i.e.* roughly 26 per cent. left undecomposed), whereas the corresponding *S*/100 solution only decreased from 12.65 to 6.9, *i.e.* 55 per cent. left undecomposed. Results at the end of 7 days lead to the same conclusion. Whereas the figure for the *S*/50 solution decreases from 25.25 to 3.35 (*i.e.* 13 per cent. undecomposed), the *S*/200 solution only decreases from 6.265 to 2.83 (*i.e.* 45 per cent. still undecomposed).

Bearing in mind the fact that the solution in which the excess acid was in the form of sulphurous acid was poorer in thiosulphuric acid at the start than those in which the excess acid was sulphuric acid, the figures show that the substitution of an excess of sulphurous for an excess of sulphuric acid has resulted in a slight increase of stability of the thiosulphuric acid; but taking into account the inaccuracy of the

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toxicity figures, this increase in stability is insufficient to affect the toxicity figures appreciably, except perhaps for the *S/20* solutions; as both of these are completely toxic under the conditions of the test, any such difference cannot show itself.

The toxicity, therefore, varies with the thiosulphuric acid content as far as can be judged from the result of this test, which, as it turns out, is admittedly not a very searching one. Even though the replacement of the excess of sulphuric acid by the excess of sulphurous acid did not result in any great change in thiosulphuric acid content, the difference in sulphur deposition (12), p. 181) shows that the equilibrium of the solutions was definitely upset in other ways. The toxicity figures following the thiosulphuric ones and not the sulphur deposition make the connection between the toxicity figures and thiosulphuric acid content the more likely to be a causal one. The decomposition of the thiosulphuric acid becomes very slow when it reaches a concentration of about *S/100* or lower. This solution is almost completely toxic in 24 hours. This fact may explain why *S/20* thiosulphuric acid remained completely toxic when the spores were exposed for 24 hours even when tested 7 days after the solution was made up, and why an *S/200* solution had approximately the same toxicity whether tested immediately after the solution was made up, 1 day or 7 days afterwards, as stated on pp. 179 and 180 of the previous paper (12).

We can test still further the idea that sulphur owes its fungicidal action in soil to the formation of thiosulphuric acid; we can see to what extent the minimum toxic concentration of thiosulphuric acid found in the laboratory fits in with the minimum effective dressing of sulphur in the field.

An exposure of Wart Disease spores to an *S/200* solution of thiosulphuric acid for 10 days results in about 19/20 being killed (12), p. 178). It has been calculated that a dressing of about 11 cwt. sulphur per acre can free the soil from disease in the ensuing season (10). We can, therefore, say that these two treatments are roughly equivalent in their effects on the disease-producing organism. 1000 c.c. *S/200* solution of thiosulphuric acid contains

$$\frac{32}{200} = 0.16 \text{ gm. sulphur.}$$

11 cwt. per acre is roughly equivalent to 0.055 per cent. on the top 9-inch soil; assuming a 20 per cent. soil mixture content, 1000 c.c. soil solution will contain $0.055 \times \frac{100}{20}$

$$= 2.75 \text{ gm. sulphur.}$$

For 1000 c.c. of solution to be completely toxic we have seen that it must contain 0.16 gm. sulphur as thiosulphuric acid over a period of 10 days, *i.e.* $\frac{0.16 \times 100}{2.75}$, or roughly 6 per cent. of the sulphur added.

It is not likely that the fungicidal action of sulphur would ever be over in so short a time as 10 days; if not, the above figure of 6 per cent. must be reduced, but against this must be placed the fact that the thiosulphuric acid has to reach each spore by diffusion in at least the minimum toxic concentration, seeing that it is continually decomposing it must start off at the source, the sulphur particle, at a somewhat high concentration.

After preparing this communication for publication the writer has read the criticisms made by Williams and Young (14) of deductions drawn in an earlier paper (12). They state: "Recent work by Roach and Glynné was interpreted by them as pointing to thiosulfuric acid as a toxic factor. However, since the thiosulfuric acid is a very unstable acid and since the condition of their experiments were such as to insure the presence of polythionic acids, the toxicity which they measured was undoubtedly that of the polythionic acids. Furthermore, the tables given by them show a notable toxicity of pentathionic acid itself when compared with other acids tested."

Facts reported on pp. 85-92 of this paper are a sufficient answer to that part of their criticism which is based on the supposed complete instability of thiosulphuric acid.

The sample of barium pentathionate used ((12), p. 188) was exceptionally pure (over 90 per cent.), and in making up the pentathionic acid solutions allowance was made for the 10 per cent. impurity which was probably water. The dilute solutions, when made up, were free from all but traces of sulphite and thiosulphate¹ and remained so the whole period of the tests (up to 10 days). The toxicities of these solutions therefore probably were a fair measure of that of pentathionic acid. The present writer cannot agree with Williams and Young's statement: "the tables...show a notable toxicity of pentathionic acid itself when compared with other acids tested" and sees no reasons for departing from the original conclusion ((12), p. 175): "The three polythionic acids were of the same order of toxicity as sulphuric acid" (see Fig. 8 in which the relevant data are reproduced). Even assuming, what may actually be true, that pentathionic acid has a slightly higher toxicity than sulphuric acid, it is definitely less toxic than thiosulphuric acid, the difference being outside the large limit of experimental error of the tests. Whatever the cause of the toxicity of acidified thiosulphate solutions it can hardly be through the formation of pentathionic acid which is of lower toxicity when compared on a sulphur basis.

That thiosulphuric is the factor chiefly, if not entirely, responsible for the toxicities of the sulphur compounds considered (over and above that due to the hydrogen ion) seems to offer the simplest explanation and the one most free from contradictory

¹ Williams and Young give the results of no such tests to establish the purity of the compounds used in their work. The writer found it necessary to prepare many samples and purify repeatedly before one even relatively free from thiosulphate was obtained.

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facts and unproven assumptions; hence it is retained unless and until a better one presents itself. The difficulties and uncertainties connected with the chemistry of sulphur compounds make anything approaching a final conclusion at this stage unjustifiable.

As already stated, the above tentative conclusion referred to Wart Disease only and does not necessarily relate to any other fungus.

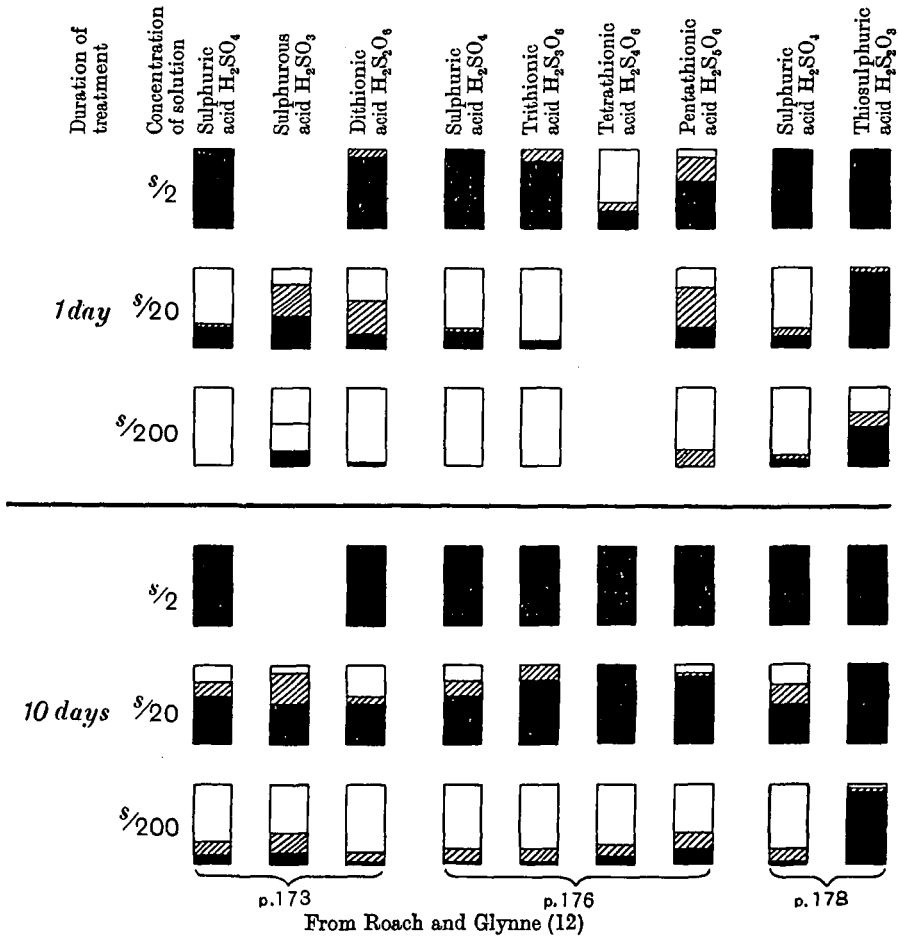



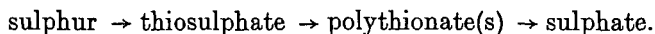


Fig. 8. Toxicities of various sulphur acids towards *Synchytrium endobioticum*.

Key to diagram:  Sporangia stained faintly and therefore alive.
 Sporangia stained to intermediate degree and probably dead.
 Sporangia stained deeply and therefore dead.

DISCUSSION.

It was suggested on p. 83 that the oxidation of sulphur to sulphate in soil-sulphur mixtures may take place in the following stages:



There seems little doubt that sulphate is the end-product under a variety of conditions. Pentathionate has been proved in the present investigation to be formed, and to accumulate, in sulphur-treated Rothamsted soil kept at 20° C., but, even if formed under the other conditions tried, it did not accumulate appreciably. These observations have been confirmed by Hobson (see Appendix). He has also shown that thiosulphate when added to soil is changed first into pentathionate and then into sulphate. Of the change of sulphur into thiosulphate, the present investigation offers no more than a mere suggestion (p. 82), but Guittonneau and Keiling (4, 5, 6, 7) proved that thiosulphate accumulated in soil-sulphur mixtures under the conditions of their experiments. Moreover, they showed that the addition of peptone greatly increased the accumulation of this substance, thus proving that the degree of accumulation is affected by the chemical composition of the soil solution.

These facts afford sufficient indications of the sensitiveness of the above series of chemical changes to physical and chemical conditions. They suggest the possibility that under the conditions of 1925 and previous years, when sulphur treatment was effective in controlling Wart Disease in the succeeding crop of potatoes, the oxidation of the sulphur may have taken place in such a way that there was a temporary accumulation of thiosulphuric acid sufficient to make the soil solution toxic to the fungus, whereas under the colder and in other ways different conditions of 1926, when the disease was apparently unaffected, there was little or no accumulation of this substance.

This explanation is the only one put forward at present at all in harmony with the facts; but it cannot be regarded as established unless and until a definite connection has been proved between the amount of thiosulphuric acid accumulating in the soil and the degree of effectiveness of the treatment.

SUMMARY.

- (1) Thiosulphuric acid has been shown to exist in a free state.
- (2) It is relatively stable in dilute solution; an *M*/200 solution is only half decomposed at the end of 1 day and an *M*/400 solution at the end of 10 days only.

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(3) This degree of stability is sufficient to account for the fungicidal action of acidified thiosulphate solutions in terms of the liberated thio-sulphuric acid.

(4) It can be calculated that it is only necessary to assume 6 per cent. of the minimum quantity of sulphur found effective against Wart Disease in the field to be in the form of thiosulphuric acid over a period of 10 days in order to account for its toxicity.

(5) Experiments of a preliminary nature carried out on sulphur-treated soil proved the formation of pentathionate in Rothamsted soil kept at 30° C., but not in Ormskirk soil kept at the same temperature, nor in either soil at the lower temperatures of 0° and 15° C.

(6) No definite evidence of the accumulation of appreciable quantities of thiosulphuric acid in the soil was obtained, but reasons are given why this negative evidence is by no means final.

(7) Chemical considerations and the work of others suggest that the pentathionate actually identified in the soil solution arose from thio-sulphuric acid formed in an early stage of the oxidation of the sulphur.

(8) The explanation of the fungicidal action of sulphur towards Wart Disease in soil in terms of the formation of thiosulphuric acid is alone in harmony with the ascertained facts.

APPENDIX.

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After Mr Roach's departure from this laboratory, I continued for a short time the experiments started by him on the decomposition of sulphur in the soil and am taking this opportunity, offered to me by him, of presenting certain results which while of interest do not merit separate publication.

Incubations of sulphur and soil gave results which agree in general with Roach's findings. Sulphate was found to be the main product of the reaction and the only intermediate compounds whose presence could be established with any certainty were the polythionates. In one experiment carried out at 30° C. the amount of polythionates lay between 2 and 7 per cent. of the sulphur added (0.1 gm. per 100 gm. soil) for a period of 30 days. During this time the amount of soluble sulphur rose from 44 to 58 per cent. of the added sulphur. At temperatures of

0° C. and 15° C. polythionates were present only in traces, less than 1 per cent. of the sulphur added.

On one occasion a water extract of sulphur-treated soil gave a blue colour with acidified potassium iodide solution containing starch, which suggested the presence of persulphate. The extract, however, gave no blue colour with an alcoholic benzidine solution, a test which is sensitive to one part of persulphate per million. An extract of untreated soil with dilute sulphuric acid released iodine in the same way, probably through dissolving a metallic oxidising constituent, as aqueous extracts gave negative results. The sulphur-treated soil was alkaline (pH 8.3), but it is reasonable to suppose that local acidity in the region of the sulphur particles brought into solution the oxidising agent. There can be no doubt that persulphate was absent.

In order to test the stability of thiosulphate in soil, sodium thiosulphate was added to Rothamsted soil, in amount equivalent to 0.1 gm. sulphur per 100 gm. soil. After 5 days' percolation was carried out and the percolate analysed 5 per cent. of the sulphur was found in the form of sulphate, 84 per cent. as polythionates and only 11 per cent. as thiosulphate. Part of the last may have been present as sulphite which was not estimated separately. The disappearance of the thiosulphate was not due to the instability of thiosulphuric acid in acid solution, as the soil was slightly alkaline in reaction. It seems probable that such a powerful reducing agent cannot exist in the presence of material as labile as soil without undergoing oxidation.

In view of the rapid disappearance of added thiosulphate it is not surprising that thiosulphate could not be detected in sulphur-treated soil. As polythionates were the main transformation product of the added thiosulphate, it seems not unlikely that the small amounts of polythionates found in sulphur-treated soil originated from thiosulphate.

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(Received October 3rd, 1929.)