The insecticidal action of sulphur
by G D Green

A THESIS Submitted to the Graduate Committee in partial fulfillment of the requirements for the Degree of Master of Science in Entomology
Montana State University

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INTRODUCTION

Sulphur alone or in combination with other substances was among the first materials recommended for use in the control of certain types of insects and plant diseases. These materials are today among the most important insecticides and fungicides. In spite of this wide and long continued use, and the great amount of work which has been directed along this line, no definite conclusion can be drawn as to the toxic property or how the toxic effect is produced.

The writer has made an effort to determine the rôle of hydrogen sulphide in the action of sulphur when applied to insects. Results of this work are presented in this paper, together with an attempt to review some of the view points expressed by other workers as to the action of sulphur and lime-sulphur solution as insecticides or fungicides.

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REVIEW OF LITERATURE

The use of sulphur in cleansing wool and clothes of moths was practiced by the ancients, being mentioned in the Old Testament. Erasmus

Darwin (1800) relates ~~the~~ a discussion on the use of a mixture of lime and sulphur in the control of aphids, mites, and scales. Elemental sulphur, usually in mixtures with inert substances as diluents, has been in continued use since the early times for the control of various insects and fungi. At the present time it is used as a specific remedy for infestations of cotton flea-hoppers, many species of mites, etc. The use of lime-sulphur and compounds of other substances and sulphur does not seem to have been so consistent. Barium-sulphur is used to some extent, but its use is not common. A combination of potassium and sulphur, known as liver of sulphur, has been used in England quite extensively against certain fungus diseases.

By far the most extensively used sulphur-containing material is lime-sulphur. Its principle use before 1889 was in stock dips, but beginning with that year it has been used as a spray for the control of scale insects. As the best known remedy for San Jose scale, its use accompanied the rapid spread of this insect over the United States. For scale insects, alone, it has been largely superseded, during the last few years, by oil sprays of various kinds. However, where there are also fungus diseases to be combatted, lime-sulphur is generally used.

Hundreds of experiments have been performed in the effort to determine the value of this material in controlling insects. Most of them have been directed toward finding the most effective proportion of lime and sulphur to use, the proper length of time to boil, the best time to apply, and the correct dilution to use. The conclusions were drawn from results of practical field experiments. Comparatively little study had been made from a chemical or physiological standpoint until about 1905.

Investigations on the chemical composition, together with tests as to the effective component have verified the methods and formulae recommended for the preparation of lime-sulphur. Standard directions, for the home preparation of the concentrate, advise boiling one hour and use of the ingredients in about the ratio of one pound stone lime, two pounds flowers of sulphur and enough water to make the finished product one gallon. From a chemical standpoint, this gives the greatest content of the components which have proven most valuable.

A considerable amount of work has been done in trying to discover the toxic property of sulphur and of lime-sulphur sprays. Many confusing conclusions have been drawn. The cause of the greater part of this confusion is likely experimenting under different conditions, misinterpretation of results, and basing conclusions on insufficient data. Most toxicity tests of the components shown to be associated with sulphur or lime-sulphur have been applied to fungi. As there is probably no essential difference in the toxic action of these materials on fungi and insects, the literature on both are included.

The first comprehensive study of the chemical composition of lime-sulphur solution seems to have been made by Haywood (1905). He thought ~~that~~ the primary reaction between lime and sulphur in aqueous solution to be as follows:

$$3\ Ca\ (OH)_2 + 12\ S = Ca\ S_2O_3 + 2\ Ca\ S_5 + 3\ H_2O.$$

As this leads to less thiosulphate and more pentasulphide than analyses show to be present, he said that probably one or both of the following secondary reactions occur:

$$3\ Ca\ (OH)_2 + 8\ S = Ca\ S_2O_3 + 2\ Ca\ S_3 + 3\ H_2O$$

and $$Ca\ S_5 + 3\ O = Ca\ S_2O_3 + 3\ S.$$

If boiling continues, more thiosulphate is formed at the expense of pentasulphide. In solution, thiosulphate changes to sulphites and these in turn to sulphates as follows:

$$Ca\ S_2O_3 = Ca\ S\ O_3 + S$$

$$Ca\ S\ O_3 + O = Ca\ S\ O_4\ .$$

These facts led Haywood to the conclusion that probably the lime-sulphur salt wash is formed as follows:

$$3\ Ca\ (OH)_2 + 12S = Ca\ S_2O_3 + 2\ Ca\ S_5 + 3\ H_2O,$$

$$Ca\ S_5 + 3\ O = Ca\ S_2O_3 + 3\ S,$$

and this sulphur $+\ Ca\ (OH)_2\ = Ca\ S_2O_3 + 2\ Ca\ S_5 + 3\ H_2O,$

to a slight extent $Ca\ S_2O_3\ = Ca\ S\ O_3 + S$

$$Ca\ S\ O_3 + O = Ca\ S\ O_4.$$

The presence of salt does not affect these reactions.

This author thinks that, in dry climates, when scales are treated with this wash, the excess lime loosens the scales and exposes the insect beneath and that the calcium pentasulphide is decomposed through the various intermediate steps into free sulphur and calcium sulphite, which are the effective agents. In wet climates, rains would wash off most of the thiosulphate and the insecticidal value would be diminished in proportion to the amount washed off.

Haywood (1905), incidentally, relates a friend, Mr. F. H. Pough, as expressing the opinion that the action of lime-sulphur was due to the gradual oxidation of sulphur into sulphur dioxide and sulphurous acid, which are the valuable constituents. Haywood doubts the oxidation being rapid enough to be of value, and credits sublimed sulphur for the supposed odor of sulphur dioxide on hot days.

Tarter and Bradley (1910) believed that the polysulphides in the solution were probably a mixture of the tetra and pentasulphide of calcium, and their analyses showed evidence of a more stable polysulphide, which was thought to be, probably, the disulphide of calcium. They decided that a considerable amount of the sulphur in the solution is very feebly combined and for practical purposes might be considered as sulphur in physical solution, and that it is not necessary for the spray to oxidize in order for free sulphur to be deposited from it. They found carbon dioxide to have a small but evident influence in the decomposition of the polysulphides, probably as follows:

$$\mathrm{Ca\,S_x + H_2C\,O_3 = H_2S + Ca\,C\,O_3 + S_{(x-1)}}.$$

These authors said that the presence of hydrogen sulphide should not be overlooked when considering the immediate action of lime-sulphur. Their conclusion is that lime-sulphur solution is a complex system subject to the influence of many varying conditions.

In 1910, Foreman published an account of work with liver of sulphur. As used at that time, liver of sulphur was not a standard product, but consisted of compounds of sodium as well as potassium. He found, in addition to sulph-hydrate, sulphides and polysulphides of either potassium or sodium, in most cases free sulphur. The oxidation products showed little or no fungicidal properties, when tried separately, in weak solution, upon the spores of *B*$\overset{o}{\text{a}}$*trytis cinerea*. Saturated solutions of hydrogen sulphide or free sulphur had absolutely no adverse effect upon the germination of spores of this fungus. He concluded from the results of his investigations that the most potent fungicidal agent in liver of sulphur is free sodium hydroxide and that potassium hydroxide was somewhat less valuable. He attributes no more effect to sulphur as a dust than to ordinary dust.

Results of a series of carefully executed experiments by Shafer (1911) indicated that the value of lime-sulphur as a scalecide lies in its strong, persistent reducing power, and its ability to soften the wax about the margin of scale insects.

Auld (1912) removed considerable hydrogen sulphide by passing purified nitrogen or oxygen through lime-sulphur solution, the evaluation increasing with rise in temperature. He thought the concentrated, commercial product to contain, generally, the polysulphides, thiosulphate, sulphite and sulphate of calcium, the polysulphides and thiosulphate in preponderance. He had no doubt that most of the sulphur in the solution is in a very loosely combined condition.

Tarter (1914), after working on this question for several years and reviewing the conclusions of Shafer (1911) and others, enumerated the following properties as the ones to which, in general, the insecticidal action of lime-sulphur seemed to be due. (1) Its power to take up large amounts of oxygen; (2) its ability to soften the newly secreted wax at the margin of scale insects, and (3) the amount of free sulphur deposited in its decomposition.

According to Chapin (1916), ignoring unessential and hypothetical intermediate compounds, the following reactions apparently occur when lime and sulphur are bailed with water:

(1) $3\ Ca\ (OH)_2 + 12S = 2\ Ca\ S_5 + Ca\ S_2O_3 + 3\ H_2O$,

(2) $1\ 0\ Ca\ S_5 + 3\ Ca\ (OH)_2 = 12\ Ca\ S_4 + Ca\ S_2O_3 + 3\ H_2O$,

and (3) $Ca\ S_4 + S = Ca\ S_5$.

Reaction 3 holds with excess sulphur and only in absence of such excess does equation 2 hold. He thought all lime-sulphur solutions subject to hydrolytic decomposition according to the equation:

$$Ca\ S_x + 2\ H_2O = Ca\ (OH)_2 + H_2S + (X-1)\ S,$$

the shifting of equilibrium from left to right increasing with rise of temperature. In case of $Ca\ S_5$ in the open air, where hydrogen sulphide can escape the final product is as follows:

$$Ca\ S_5 + 3\ H_2O = Ca\ S_2O_3 + 3\ H_2\ S.$$

Doran (1922) found that commercial lime-sulphur in dilutions of one to ten did not prevent the germination of the conidia of Venturia inequalis in the absence of sulphides. In a single experiment he obtained absolute control of this fungus when dusted with sulphur and exposed to air, and none in the absence of oxygen. A rise in temperature or a longer time of exposure increased the toxicity of sulphur in his experiments.

Thatcher and Streeter (1925) suggest that elemental sulphur, as deposited in the decomposition of lime-sulphur or applied in dusts, is a solid, non toxic material, its toxic effect being produced only after it has been changed into some gaseous form. Conclusions of other workers, mentioned, attributed the fungicidal effect to sulphur dioxide produced by oxidation of sulphur in moist air. It is reported that other workers, by use of pure sulphur dioxide, have failed to verify these conclusions. Two possible and different fungicidal effects of sulphur containing materials seemed obvious to these authors. One is the specific toxic effect of calcium polysulphide or similar compounds which can exert their effect only so long as these materials remain as such on the tree and against only the disease with which they come in contact. The second effect is that due to the gaseous substances produced by the oxidation of free sulphur which continues as long as there is enough sulphur remaining on the foliage to produce a sufficient amount of the toxic gas to kill the particular pest in question.

In an ordinary meeting of the Association of Economic Biologists, October 20, 1925, a long discussion on the fungicidal action of sulphur was carried on. The following points are recorded from that discussion, Anon (1926):

Professor B.T.P.Barker, in discussing the work at Long Ashton, said: at the outset it was recognized that attention could not be confined to the action of uncombined sulphur. Hence, the action of spray fluids of the polysulphide type were examined and the conclusion arrived at that their protective fungicidal action must be attributed to the sulphur which is rapidly produced by the decomposition of the polysulphides after the application of those sprays to the plant. Varied experiments demonstrated that the toxic effect could be exerted across a space. When air, in a greenhouse treated with a coating of sulphur on the hot water pipes, was filtered through cellulose pads or passed through bent glass tubes, it was found to be non-toxic and particulate sulphur was deposited on the pads and in the tubes. Air, from the same source, not so treated was toxic. The results were interpreted to mean that sulphur travels through the air in particulate and not in gaseous form, and that it is in the form of elemental sulphur and not a compound. Three alternative views were deduced as to the action of this particulate form. (1) The possibility that particulate sulphur is dissolved in the film of moisture surrounding the fungus. This view was dispersed by the insolubility of particulate sulphur. (2) Sulphur directly toxic in particulate state, a view unsupported by positive evidence. (3) The third alternative action presented was that of sulphur compounds formed from particulate material.

The substances given consideration under the third viewpoint were sulphur dioxide, sulphur trioxide, sulphuric acid, pentathionic acid, hydrogen sulphide, and other sulphides. No evidence of the formation, in adequate strength, of sulphur dioxide, sulphur trioxide, or sulphuric acid eradicated these substances. Long Ashton Experiment Station work did not confirm the pentathionic acid theory of Young, and the fact that Young found sulphur toxic outside the limits of hydrogen ion concentration stated as necessary for the stability of pentathionic acid seemed enough to put it out of account. The production of hydrogen sulphide when green leaves are treated with sulphur was mentioned.

Mr. Tattersfield of Rothamsted considered that with respect to the fungicidal action of the polysulphides, it would definitely appear as if the direct action were due to alkalinity and the action of the polusulphides as such, but that the prolonged effect and the action at a distance were in some way bound up with the sulphur particles deposited from the solution.

Goldsworthy (1928) believed the fungicidal properties of polysulphide solutions dependent, at least in part, upon the soluble sulphide content. He found sulphur to be deposited in germ tubes, and spores to be arrested in their development, and concluded the lethal factor to be the oxidation of sulphide to sulphur. This was thought to destroy the poising material of the oxidation-reduction system to a point beyond which recovery is impossible.

Goodwin and Martin (1928) found that by heating glass wool filters or glass tubes and drawing air, from sulphur treated greenhouses, through them that no particulate sulphur was deposited and that the air did not lose its toxic property. This proved that the sulphur was gaseous. The removal of the volatile agent by passage through a cooled filter was taken as proof that it was neither sulphur dioxide or hydrogen sulphide, but was elemental sulphur. Condensation of volatilized sulphur on cooled surfaces was given as the reason for the deposition of particulate sulphur mentioned by Barker.

Roach and Glynne (1928) concluded, tentatively, that thiosulphuric acid was the principal toxic substance associated with sulphur. Other acids normally associated with sulphur were found to have about equal toxicities and this was attributed to the hydrogen ion concentration.

Williams and Young (1929) found ordinary ground roll and flowers of sulphur to yield varying amounts of the toxic factor, depending upon the reaction, oxidation, temperature, and moisture. The addition of oxidizing agents enhanced the toxicity greatly. From tests with all the acids known to be associated with sulphur, they report only the polythionic, (tetra and penta), acids to be toxic. Samples of sulphur freed from these acids were non-toxic. The toxic factor was said to be destroyed by strong acids and alkalies.

The main criticisms directed against the conclusions of Williams and Young seem to be the following: (1) Their failure to explain their method of isolation or preparation of the pentathionic acid used. This, with the known difficulty of preparing this acid free from impurities, and its variable stability under different conditions, seem to be taken as evidence that they could not be definitely sure of the compound with which they were working. (2) Their statement that pentathionic acid was stable only within the pH limits of 4.2 - 5.4 and then giving results of fungicidal activity of sulphur below a pH of 3.2 and 2.4.

According to DeOng and Huntoon (1929), the insecticidal action of lime-sulphur solution is due, first, to the reducing or oxygen absorbing power of the sulphide, and secondly, to the action of free sulphur.

Tucker (1929), by a series of experiments, found sulphur to be deposited on the sides of containing vessels at ordinary temperatures. At the temperature of boiling water, only traces of hydrogen sulphide or the products of oxidation of sulphur could be detected, leading to the conclusion that this deposited sulphur was the result of condensation of sublimed sulphur. The fact that the increased rate of action with succeeding rises in temperature of 10 °C. corresponded with the rate of physical rather than chemical action strengthened this conclusion. In the control of certain plant diseases, the effectiveness of sulphur increases at the same rate, with rises of temperature, as the rate of sublimation. The value of sulphur as a fungicide, Tucker thought, depended primarily upon the sublimation of sulphur as such.

The results of various workers who have found sulphur to act across a space confirm this conclusion.

Further proof of the action of sulphur through a distance is the experimental results of McGregor (1927) with the tarnished bug, *Lygus elisus*. These insects, confined in screen cages 12 to 24 inches above the ground, over sulphur dust liberally applied to the surface of the soil, at temperatures of from 102°F. to 116°F., died within 30 minutes. The controls similarly treated, except the sulphur, suffered no mortality.

O'Kane and Conklin (1930), from results of laboratory tests, concluded that hydrogen sulphide is not given off from lime-sulphur solution in sufficient quantities to account for the toxicity of that material. They found no trace of sulphur dioxide being evolved.

No method of analysis, yet suggested, will show the concentration of hydrogen sulphide in the immediate vicinity of an insect in contact with sulphur or lime-sulphur solution. The chances are that it is many times greater than analyses show, and it cannot be lower.

Wilcoxon and McCallan (1930) found the toxicities of sulphuric and pentathionic acids to be equal, within experimental error, and hydrogen sulphide to be 6 to 200 times as toxic as these acids under varying conditions. Water extracts of sulphur gave no control. The toxicity of commercial dusting sulphur, after the removal of acids associated with it, was fully as high as the same material without such treatment. It was concluded that pentathionic acid is not a factor of importance in the fungicidal action of sulphur.

McCallan and Wilcoxon (1931) present a large amount of evidence that living tissues in contact with sulphur are able to bring about its reduction to hydrogen sulphide. In their tests on 26 species of plants of 16 families, hydrogen sulphide was produced in every case, there being, however, marked variation in the rate of production by different species.

Spores from some 27 species of fungi produced hydrogen sulphide when treated with sulphur, the rate in this case, also, varying with different species. The effect of temperature indicates a reaction enzymatic in nature. They found ~~that~~ within the pH range of 4.0 to 8.0, the rate of evolution to be independent of hydrogen ion concentration. A concentration of 0.2 mg. per liter of hydrogen sulphide was completely toxic to Venturia inaequalis where with Botrytis spp. of the cinera type, there was 3.8 percent germination at a concentration of 20 mg. per liter. The order of sensitivity of 8 species of fungi to hydrogen sulphide and to sulphur was exactly the same. There was a positive correlation between the sensitiveness of a species to hydrogen sulphide and its ability to produce that gas when treated with sulphur.

To give a clearer presentation of the point of view of McCallan and Wilcoxon (1931) their "Discussion" shall be quoted. "In presenting any theory to explain the fungicidal action of sulphur cognizance must be taken of the following experimentally determined facts. Leaves and spores of all species of plants tested produce hydrogen sulphide when in contact with sulphur. The production of hydrogen sulphide by spores varies according to the species and is directly proportional to the number of spores. The optimum temperature is 35°C. and inhibition takes place at 55°C. but the production occurs over a relatively wide pH range. The maximum rate of production at 30°C. is reached in about 3 hours. Actual contact between spores and sulphur is not necessary as action will take place through a collodion membrane, the hydrogen sulphide being produced on the spore side of the membrane and not on the sulphur side. The action also takes place across an air space.

"The action of spores on the sulphur is primarily a reducing one. Hydrogen sulphide is extremely toxic to fungous spores, much more so than any other sulphur acid. The order of sensitivity of the spores of different species to sulphur and to hydrogen sulphide is identical and the sensitive spores produce more than one toxic unit of hydrogen sulphide, while the resistant ones produce considerably less than one toxic unit. Finally, compounds such as glutathione, which contain the -S H group, readily react with sulphur producing hydrogen sulphide at ordinary temperatures, and such compounds have been shown to exist in fungous spores.

"The following interpretation of these facts as related to the fungicidal action of sulphur is offered. Sulphur in the vicinity of fungous spores, by reason of its vapor pressure, gives off sulphur vapor which diffuses into the spores. Here reduction takes place within the spores with hydrogen sulphide as a final product. The reaction is enzymatic in nature and is probably concerned with - S H compounds. The toxic product, hydrogen sulphide, being produced in intimate contact with the living cell is able to exert its maximum effect. It is not believed that the hydrogen sulphide produced from the leaves in the open effects the spores, nor that the hydrogen sulphide produced by one spore has much effect on another spore at a distance. Each individual spore, therefore, by reason of its ability to reduce sulphur to hydrogen sulphide, is thus instrumental in bringing about its own death."

EXPERIMENTAL METHODS AND RESULTS

The Production of Hydrogen Sulphide by Sulphur Treated Insects

In order to determine the role of hydrogen sulphide in the toxicity of sulphur to insects, the first step necessary was to test their ability to produce hydrogen sulphide when treated with sulphur. The method used was as follows: The samples of insects or material to be tested were placed in similar vials, a thorough dusting of flowers of sulphur applied, and the vials tightly stoppered with corks from which strips of filter paper moistened with saturated lead acetate solution were suspended as indicators. The vials were then placed under the desired conditions of temperature for the duration of the experiment, observations being made at frequent intervals. The production of hydrogen sulphide was shown by a black precipitate of lead sulphide on the filter paper. These tests were run with live insects, garden slugs, field spiders, clover mites, insects with intestinal tract removed, the intestinal tract itself, ground up insect tissue, etc. The results were positive in every case, and varied with different species. Controls with these same materials, under the same conditions except sulphur, also gave positive results except with most of the intestinal tracts and tent caterpillar larvae. In case of the controls, as may be seen in Table I, the production was much slower than with treated material. No hydrogen sulphide was produced in controls with sulphur alone or as a paste. Most of the species used are not affected by sulphur and Colorado potato beetles seemed to fare better in sulphur than in the untreated checks, yet they produced hydrogen sulphide readily.. A possible explanation of this fact is the adverse action of the hydrogen sulphide produced upon the putrefying bacteria associated with the insect.

The production of hydrogen sulphide by untreated material results from the decomposition of proteins by putrefying bacteria. Almy (1925) states that hydrogen sulphide is one of the end products resulting from the action of many varieties of bacteria on organic material containing protein. This author credits Rettger[1] as stating that hydrogen sulphide is, no doubt, one of the first substances which is split off from t the protein molecule during putrefaction.

A quantitative determination of the amount of hydrogen sulphide produced by insects treated with sulphur is, by any method suggested heretofore, impossible. The amount produced is small, and as it is produced in the tissue where it is combined with and adsorbed by various substances, on the cuticula, and walls of the container, any quantitative estimation would be unreliable.

A method decided upon which would seem to give a somewhat comparative quantitative estimation is given below. The blackening of the filter paper indicator, as hydrogen sulphide was produced, always began at the bottom and proceeded gradually upward, so approximate standards were made and applied to the amount of the paper blackened after a certain time. From the application of these standards, the results shown in Table I were obtained. The results in Table I are averages of from 1 to 5 or 6 tests. If no blackening occurred, 0 or no test was recorded; from

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1 - Rettger. Jour. Biol. Chem. 2:71, 1906-7.

the slightest darkening at the tip to 1/3 black was recorded as X or light test; 1/3 to 2/3 black as medium test or XX; 2/3 to fully covered as XXX or heavy test, and very black as XXXX or very heavy test.

It was thought that by injecting lead acetate solution into the body tissue of clear skinned species, and treating with sulphur, that possibly the precipitation of black lead sulphide would show the progress of hydrogen sulphide production in the body.

Large larvae of white grubs and round-headed wood borers were selected for the test. The lead acetate solution, however, killed the larvae soon after injection and the experiment was not successful. Some of the larvae were placed in vials, treated with flowers of sulphur, and kept overnight. Upon injection the following morning, some precipitate was shown near the point of injection. This precipitate was not very noticeable, and was not shown over all parts of the body. These larvae do not contain much firm tissue, and most of the solution escaped through the small hole made by the hypodermic needle as soon as it was removed. This probably, in part, accounts for failure of other parts of the body to show a precipitate.

A white grub larvae, injected with lead acetate and placed in a concentration of hydrogen sulphide of 5 milligrams per liter, immediately turned black all over.

These species are not very much affected by sulphur, and do not show the production of hydrogen sulphide very readily. The hydrogen sulphide is produced in the tissue and its production is very slow. Any combination with and adsorption by substances in the tissue takes place as rapidly as it is produced until such combining and adsorbing capacities

TABLE I. SHOWING THE PRODUCTION OF HYDROGEN SULPHIDE BY SULPHUR TREATED INSECTS

Material	Treatment							
	Sulphur				No Sulphur			
	1 hr.	4 hrs.	24hrs.	72hrs.	1 hr.	4 hrs.	24 hrs.	72 hrs
Colorado potato beetle, *Leptinotarsa decemlineata*, Adults	0*	xx	xxx	xxxx	0	0	x	xxx
" " " " "(less int.tract)	0	x	xxx	xxxx	0	0	x	xxx
" " " " "(Ditto ground up)	0	x	xx	xxxx	0	0	x	xxx
" " " " "(int. tract)	0	x	xx	xxx	0	0	0	0
" " " " " larvae	0	x	xx	xxx	0	0	x	xxx
" " " " "(less int.tract ground up)	0	x	xx	xxxx	0	0	x	xx
" " " " "(intestinal tract)	0	x	xxx	xxx	0	0	0	0
Garden slugs, *Agriolimax agrestes*	0	x	xx	xxx	0	0	0	xxx
" " " " (ground up)	0	x	xxx	xxxx	0	0	x	xxx
Grasshoppers, *Melanoplus bivittatus*	0	0	x	xx	0	0	0	x
" " " (intestinal tract)	0	x	xxx	xxx	0	0	0	0
" " " (less int. tract)	0	x	xxx	xxx	0	0	x	xx
" " " (" " ",ground up	0	x	xxx	xxx	0	0	x	xx
Imported cabbage worm, *Pieris rapae*, larvae	0	0	xxx	xxx	0	0	x	xx
" " " " " (less int, tract)	0	0	xx	xxx	0	0	x	xx
" " " " " (int. tract)					0	0	x	xx
" " " " " (excreta)					0	0	0	x
Mites, clover, *Schizotetranychus pratensis*	0	x	xxx	xxx	0	0	xx	xx
Sugar beet webworm, *Loxostege sticticalis*, larvae	0	x	xxx	xxx	0	0	x	xx
Tabanid larvae	x	x	xxx	xxx				
Tent caterpillar, *Malacosoma* spp., larvae	0	x	xx	xxx	0	0	x	xx
" " " " (ground up)	0	0	x	xx	0	0	0	0
Tribolium confusium, adults	0	0	x	xx	0	0	0	0
Sulphur paste, different dilutions as check	0	0	x		0	0	0	
	0	0	0	0				

*- 0 no test, x light test, xx medium test, xxx heavy test, xxxx very heavy test.

are exhausted. Until there is hydrogen sulphide present in excess of that combined or adsorbed, no lead sulphide will be precipitated upon injection with lead acetate. In view of these facts it is not surprising that no great amount of blackening was shown, in case of sulphured larvae.

The Effect of Temperature on the Reaction

The rate of hydrogen sulphide production gradually increased from room temperature, about 22°C., up to 37°C., which was the highest temperature used. The increase was not as great as expected, which can be explained by the reasons given for making quantitative determination impossible.

The Effect of Hydrogen Ion Concentration on the Reaction

Tests were run on yeast in solutions of pH value from 1 to 12. The results were so nearly the same that ~~until~~ it seems that the hydrogen ion concentration does not effect the reaction within these limits. These results are in agreement with those of McCallan and Wilcoxon (1931) on this point, interpreted by them as further evidence in favor of the conclusion that the production of hydrogen sulphide goes on inside the organism.

No sulphur sensitive species were available in sufficient quantities to be used for these tests. Only enough mites were secured to run one test on a small scale. Species which are controlled by sulphur might produce hydrogen sulphide much more readily than most of the species used.

Hydrogen Sulphide Production by Lime-Sulphur

In a series of tests, under the conditions outlined for the experiments with sulphur, hydrogen sulphide was evolved readily from dry lime-sulphur, alone, as removed from commercial containers. Solutions of this material, made by adding 2 teaspoonsful to a pint of water, produced hydrogen sulphide in appreciable quantities, when applied to insects or alone. The residue from this solution, after evaporation to dryness in the open air, also, produced this gas alone or when added to insects. The controls, with the material alone, produced hydrogen sulphide in each case but not as rapidly or in as great quantities as when applied to insects. Colorado potato beetles were used in these tests.

The Toxicity of Sulphur to Some Species

Realizing that the species available for experiment were not capable of being controlled by sulphur, it was desirable to learn whether they were at all affected by this substance. The methods used and results obtained follow.

Four male and 4 female grasshoppers, (Melanoplus bivittatus), were placed in a large vial, thoroughly dusted with flowers of sulphur, and tightly stoppered. An equal number of as near the same size and activity as possible, were placed under exactly the same conditions, except sulphur treatment as checks. After 10 hours all specimens, both treated and untreated, had ceased all activity and were apparently dead. They were then taken out of the vials and placed separately in screen topped containers for observation. Within 5 minutes two of each had recovered sufficiently to jump against the tops of the containers. At the end of 12 hours, all of the untreated individuals had apparently recovered completely. At this time 2 of the treated females were apparently dead, and 2 had only recovered enough to hold on to the finger when picked up. The weakened females never recovered. Under the conditions of the experiment, sulphur is apparently somewhat toxic to this species.

Colorado potato beetles given the same treatment seemed to be slightly weakened, but after 4 hours in the open air recovery was complete.

Subjected to the same conditions for 24 hours, _Tribolium confusum_ showed no ill effects. The same was true for _Sitophilus oryzae_.

The Relationship of the Glutathione Content of a Species to the Ability of that Species to Produce Hydrogen Sulphide from Sulphur.

The ability of glutathione, a tripeptide, to reduce sulphur to hydrogen sulphide is well established. This tripeptide, of glycine, glutamic acid and cysteine, has been studied quite exhaustively by Hopkins, Tunnicliffe (1925), Mason (1931), Meldrum and Dixon (1930), and others. This substance is widely distributed in living organisms. To determine the correlation between the glutathione content of various species and their ability to produce hydrogen sulphide when treated with sulphur, might throw some light on the nature of the reaction. Some effort was directed along this line. The method of Fink (1927) with minor modifications was used in the determinations. The procedure is as follows:

Approximately 10 grams of insects or material used was weighed to four decimal places, cut up, placed in a glass mortar with a little sand and a few cc. of a 10 percent trichloracetic acid solution, and ground up thoroughly. This mixture was then placed in a flask, and enough additional acid solution added to cover the material. The flask was then tightly stoppered and left for about 10 hours. The material was filtered, the residue placed back in the flask, more acid added, and left for 5 hours when it was filtered again. The filtrates were combined and the following determination run. Five cc. of the filtrate was drawn out, placed in a beaker and titrated with approximately 0.0 1 N iodine solution, using starch solution for an indicator. The stock iodine solution was standardized before each titration by titrating with standard sodium thiosulphate solution. The chitin content was estimated by removing the tissue and other foreign material from weighted samples with 10 percent sodium hydroxide solution. The insects were cut open, placed in the solution, left overnight, removed, washed thoroughly, dried in a sulphuric acid desiccator and the remaining material calculated as the chitin content. The percentage of water was determined by dehydration through a series of from 6 0 to 9 0 percent alcohol and a sulphuric acid desiccator.

The amount of glutathione contained in milligrams per gram of tissue was calculated on the basis, as used by Fink (1927), that 1 cc. of 0.0 1 N iodine is equivalent to 2.5 milligrams of glutathione. The results are shown in Table II. Each result in this table is the average of from 1 to 4 tests.

TABLE II. GLUTATHIONE CONTENT OF SOME INSECTS, SLUGS, ETC.

Material Tested	Average, mg. per gm.
Leptinotarsa decemlineata, adults	2.42
" " larvae	1.90
Melanoplus bivittatus	1.10
Slugs, Agriolimax agrestes	1.46
Fleischmans yeast	6.83
Corn, Zea mays	1.13
Lettuce, Lactuca sativa	1.76
Potatoes, Solanum tuberosum, tubers	0.47
Lilac, Syringa vulgaris	0.82

It is realized that these results are not altogether accurate for several reasons, some of which follow. First, the experimental procedure was not such as to produce accurate results. Second, the difficulty in determining exactly the end point in the titration makes the results questionable. Third, the likely presence of -S H groups besides those in undissociated glutathione would make the method unreliable. However, as similar procedure was followed in all the determinations, it is thought that the results might answer for comparative purposes.

From the results obtained, it appears that there might be some positive correlation between the glutathione content of a species and its ability to produce hydrogen sulphide from sulphur. However, the limited number of determinations and the method used do not permit definite conclusions.

The Effect of Sulphur on the Catalase Activity of Melanoplus bivittatus.

Shafer (1915) found that insecticides affect to a greater or lesser extent the enzymatic balance between the oxidases, reductases, and catalases. As equipment was available with which the effect on catalase could be measured, the following tests were made. Four male *M. bivittatus* were placed in vials and thoroughly dusted with flowers of sulphur. Four males of the same species, as near the same size as the treated ones as possible, were placed in similar vials under the same conditions except dusting. All vials were tightly stoppered for about 10 hours, when the stoppers were slightly loosened to prevent suffocation. After 24 hours, each grasshopper was weighed, ground for 5 minutes in a mortar, and treated with excess hydrogen peroxide in a gas measuring device. The basis of comparison was the average amount of free oxygen liberated per second from 1 gram of pulp from treated and untreated individuals. The evolved gas was measured in a gas burette over water at 20°C. The average amount of oxygen liberated per second from 1 gram of pulp of untreated *M. bivittatus* was 2.7 cc. compared with 2.31 cc. per second per gram for sulphur treated individuals.

These results indicate that the activity of the catalase in the insect's body may be somewhat inhibited by treatment with sulphur. However, this inhibition is probably not enough to upset the enzymatic balance to any important extent.

The Toxicity of Hydrogen Sulphide to Insects

The toxic component or property of sulphur must be associated with that element under conditions as found when it is applied in the field. The component or property to which the greatest value is attributed, other things being equal, must prove the most toxic when tested under controlled conditions. To determine the status of hydrogen sulphide as a toxic agent in the action of sulphur, toxicity tests were run with that gas and the "median lethal concentration" calculated.

Strand (1930) showed that as a basis for comparison, the "median lethal concentration or dosage" ~~was~~ is by far the best method of expressing toxic concentrations. He also showed that if the concentration is varied, time and temperature should be held constant. Five hours was selected as the most convenient time of exposure. By "median lethal concentration" is meant the concentration of the substance required to kill 50 percent of the organisms in consideration under the conditions of the experiment. In this case, it means the concentration in milligrams per liter necessary to kill 50 percent of *Tribolium confusum* at 25°C. under 5 hours exposure. As past history very likely affects the physiology of insects with respect to adsorption and diffusion of gases, and other factors related to toxicity, it is necessary that the specimens used in comparative tests be reared under similar conditions. The insects used in this case were taken from a culture of confused flour beetles which were kept at approximately 25°C.

The experimental procedure was as follows. Bolting cloth cages containing about 30 *T. confusum* were suspended about 2 or 3 inches from the bottom of 6.4 liter Erlenmeyer flasks, which were then stoppered with glass stoppers. Each of these stoppers was constructed with two glass tubes through it, which were provided with stopcocks. A partial vacuum was created in each flask by drawing out all the air one could by mouth. One of the stopcocks was attached to a gas burette. The desired concentration of hydrogen sulphide was drawn into the flask, then the other stopcock opened which allowed air to rush in until the pressure inside equalled that of the atmosphere. The flasks were then placed in a large temperature cabinet at 25°C. At the end of 5 hours the cages were removed, and the number of dead and live insects recorded. A final count was made 72 hours after removal. The concentration in milligrams per liter and the percent mortality was calculated for each test. Forty-two tests were run with 2 cages or 60 insects per test. The percentage mortality in the checks was so small that it was neglected. From the results obtained, the toxicity curve, shown in Figure 1, was made by plotting concentration in milligrams per liter against percentage mortality and drawing the best curve through the points. The "median lethal concentration" of hydrogen sulphide for T. confusum as calculated from this curve was very near to 3.60 mg. per liter. The minimum lethal concentration was about 6.8 mg. per liter or 0.5 percent by volume.

This is 1.1 mg. per liter lower than Strand (1930) obtained for chloropicrin and 4.33 mg. per liter lower than he obtained for ammonia. It is a little over 9 times as toxic as ethylene dichloride, 16.9 times as toxic as carbon disulphide and 1/6 as toxic as hydrocyanic acid gas, when compared with the results of that same author.

A few tests with the rice weevil, Sitophilus oryzae, show the median lethal concentration of hydrogen sulphide for this species to be near 6.8 mg. per liter. The different species, therefore, vary in their resistance to this gas.

No consideration was made for adsorption or diffusion. The gas used was a commercial product and considered as 100 percent pure. The concentration was, therefore, probably, lower than that calculated.

Tests show both *T. confusum* and *S. oryzae* to be very slightly if at all affected by sulphur. It is very likely, therefore, that toxicity tests with hydrogen sulphide on extremely sulphur sensitive species, as red spiders or cotton fleahoppers, would show total mortality at much lower concentrations than necessary for the species tested. It is not at all difficult to imagine such a concentration in or right around an insect's body when in contact with sulphur, even in the open air.

Unfortunately no sulphur sensitive species were available in sufficient quantities for toxicity tests. Such being the case, it was impossible to correlate, any further, sulphur sensitivity with the toxicity of hydrogen sulphide.

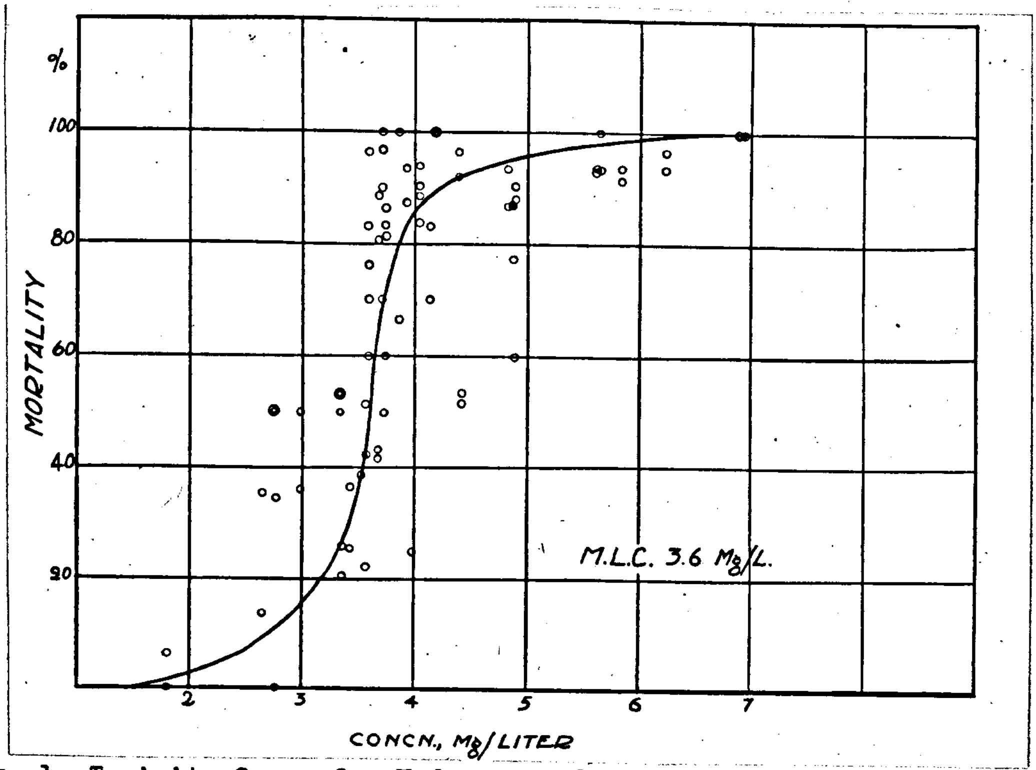

Figure 1. Toxicity Curve for Hydrogen Sulphide From Which the Median Lethal Concentration for the Confused Flour Beetle Was Calculated.

DISCUSSION AND CONCLUSIONS

Some facts to be considered in drawing a conclusion as to the toxic action of sulphur to insects are as follows.

Some detrimental effect of lime-sulphur solution is possibly due to the imprisonment of the young under the wax covering of the mother scale. Shafer (1911) showed the freshly secreted margins of these scales to be softened, in some cases, causing them to adhere to the bark.

Cooley (1910), however, shows the real effect of lime-sulphur to be exhibited on the young scale insects, immediately after hatching, often a month or six weeks after application of the spray. Studies on the chemical composition of lime-sulphur show the tendency for free sulphur to be deposited as the final product. By the time the young scales hatch, practically all the sulphur in the dormant or delayed dormant spray has been deposited as the free element.

The consensus of opinion as to the action of lime-sulphur points to some property of the element itself.

McGregor (1927), working with insects, and McCallan and Wilcoxon (1931), and others, working with fungi, have shown that the action can take place across a space.

The fact that the toxic action can be exerted across a space leads to the conclusion that sulphur enters the body of the insect as gaseous or sublimed sulphur.

McCallan and Wilcoxon (1931) showed sulphur sensitivity of eight species of fungi to correspond perfectly with the toxicity of hydrogen sulphide to the same species. They concluded from their results that each fungus spore brings about its own death when treated with sulphur by reducing the element to the extremely toxic hydrogen sulphide.

Particulate sulphur is usually thought of as a rather inert substance, but the vapor has greatly increased surface and other physical differences making the possibility of a much more active material.

As to the toxic action of sulphur, there is no doubt that death in most cases is brought about by the portion that enters the insect's body in the gaseous state, and evidence favors the conclusion that the fatal result is caused by the hydrogen sulphide which is produced from its reduction inside the body.

SUMMARY

A somewhat extensive review of viewpoints dealing with the toxic action of sulphur and lime-sulphur solution, presented in the readily available literature, is included.

The leading reports on chemical investigations of lime-sulphur solution are reviewed.

The readiness with which sulphur is reduced to hydrogen sulphide by living insects is shown.

Experiments are described which show this gas to be highly toxic to sulphur resistant species. The inference is made that it would likely prove much more toxic to sulphur sensitive species.

Results of other workers show that the toxic action can be exerted across a space.

It is concluded that the portion of the sulphur which does most of the damage to insects enters the body as gaseous sulphur. Whether it produces its toxic action upon insects in this form or is reduced to hydrogen sulphide which brings death, can only be inferred from indirect evidence.

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