There is urgent need for a fungicide which may be used effectively, without danger of injury, on a variety of plants under diverse conditions.

Sulphur has long been known to have fungicidal properties. Previous to 1880 it was almost the only fungicide in use, doubtless due, in large part, to its abundance and low cost. In the years 1848, 1852, and 1853, during the serious outbreaks of the grapevine mildew in France (Bourcart, '13) it played an important part as a fungicide. The diversity of the forms and compounds of sulphur has had an important bearing upon the use of this substance as a fungicide. The application of a mixture of lime and sulphur for the control of grape mildew was recorded as early as 1833 (Scott, '08), before the use of fungicides became general. Lime sulphur is at present the form of sulphur most widely employed and it ranks high among the standard spray mixtures. However, the use of this spray has been limited to a certain extent because of the caustic effect upon the foliage of certain plants, due to the soluble sulphide content. Serious burning is often reported from its use on apples, which are among those plants least susceptible to this type of injury. The application of this preparation to stone fruits is usually considered unsafe, and it cannot be satisfactorily used on the small fruits and many other plants.

A number of theories have been proposed respecting the toxic constituent of the different forms of sulphur, and until recently the problem was a subject of considerable speculation. A complete review of the earlier investigations has been given in a recent paper by Young ('22). This investigator demonstrated that regardless of the form of sulphur employed as a fungicide, whether as a compound or as uncombined sulphur, the lasting toxicity is due to an oxidation product of the sulphur itself. He further proved that the toxic substance is liberated most

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1 A fellowship established by the Crop Protection Institute for the investigation of sulphur as a fungicide.
rapidly from sulphur in a very finely divided state, that is, from colloidal sulphur.

**Experimental**

Since the experimental evidence gained from laboratory tests, up to the present time, has shown that colloidal sulphur has greater fungicidal value than other forms of sulphur, it was thought important to study the methods of its preparation. The primary object was to perfect a method or methods which would serve as a basis for commercial production of colloidal sulphur. After studying a variety of preparations, those materials which indicated sufficient promise were thoroughly tested for injury on the foliage of a variety of plants growing in the greenhouse. The plants used in these tests were the following: peach, potato, tomato, squash, cucumber, cantaloupe, geranium, tobacco, sweet-potato, and rose. Several of these plants were found to be very susceptible to injury by certain sulphur compounds. In those cases in which injury resulted a further study was made in an effort to eliminate the injurious property. The toxicity of the materials was then tested, using the percentage of germination of spores of a variety of economic fungi as indicators. The Van Tieghem cell or the modified hanging-drop culture method used by Young ('22) was employed in this work. The following organisms were used: *Botrytis Allii*, *B. cinerea*, *Colletotrichum Gossypii*, *Glomerella cingulata*, *Gloeosporium venetum*, *Macrosorium sarcinaeforme*, *Sclerotinia cinerea*, and *Ustilago Hordei*. The spores were taken from cultures 10–15 days old, grown on dextrose-potato agar prepared by the method of Duggar, Severy, and Schmitz ('17), except in the case of *Ustilago Hordei*. The spores of the latter organism were obtained from smutted barley heads, freshly collected in the field.

The culture solution used in the hanging drops and in which the sulphur materials were suspended, except in cases otherwise indicated, was a slightly buffered mixture prepared according to the method of Karrer and Webb ('20) and of Young ('22), as follows: Stock solutions of M/5 mannite in M/10 phosphoric acid and of M/5 mannite in M/5 sodium hydroxide were prepared. Equal quantities of the M/5 mannite-M/10 phosphoric acid were
placed in each of ten flasks and successively increasing proportions of M/5 mannite-M/5 sodium hydroxide were added. The flasks were plugged with cotton, sterilized at 15 pounds pressure for 15 minutes, and allowed to stand for a few hours. Titrations made by the colorimetric method (Clark,'20) showed the mixture to have the following range of hydrogen-ion concentrations: \( \text{pH} \) 2.4, 3.4, 4.2, 5.0, 5.4, 5.8, 6.2, 6.8, 7.4, 8.4.

**THE PREPARATION OF COLLOIDAL SULPHUR FROM SODIUM THIOSULPHATE AND SULPHURIC ACID**

It has been shown by Young ('22)^ and others that when a saturated solution of sodium thiosulphate (hypo) is mixed with concentrated \( \text{H}_2\text{SO}_4 \), a colloidal sulphur is formed having extremely small particles. In order to free the sulphur from the acid solution, it was coagulated with concentrated \( \text{NaCl} \) and centrifuged out of suspension. The coagulum was then peptized with water. By repeating the coagulation, centrifuging, and peptizing, a pure sample of colloidal sulphur is obtained—except for traces of the salt. This form of colloidal sulphur was found to have a high fungicidal value, but the method of preparation proved to be impractical for its production on a commercial basis.

There remained one other possible method of remedying the acid-hypo mixture, so that it might be used as a spray; namely, to neutralize it with an alkali. Young ('25) also encountered difficulties in this process. He first used \( \text{NaOH} \) to neutralize his hypo-acid mixture and found this alkali to be unsatisfactory because the concentration of the electrolyte soon precipitated the soluble sulphur. He later used lime sulphur to neutralize the mixture and prepared the material as follows: Fifteen gallons of saturated hypo were slowly stirred into 5 gallons of concentrated \( \text{H}_2\text{SO}_4 \) in a wooden barrel. The mixture was filtered through a fine screen and the filtrate neutralized with lime sulphur diluted one to three with water. During the addition of the lime sulphur, which was slowly effected, a further quantity of 3 gallons of concentrated hypo was introduced. Analysis of the final mixture

^ Young gives a review of the earlier investigations upon this method of preparing colloidal sulphur.
showed that 1 gallon contained 10 ounces of soluble sulphur. He suggested that if the mixture could have been centrifuged at this point it would have made an excellent spray. As a result of the conditions, however, the soluble sulphur soon precipitated.

When the hypo and H₂SO₄ are mixed according to the method just described, there is a large quantity of insoluble sulphur formed which is thrown out in the form of a gummy mass. This insoluble mass must be filtered off, as has already been indicated, before further procedures. It appeared logical to suspect that this waste could be prevented to a large extent by the introduction of certain glutinous colloids which might be assumed to act as protective colloids. Likewise, the introduction of such colloids might be assumed to exhibit further protective action by preventing the precipitation of the soluble sulphur when electrolytes are added to neutralize the mixture.

E. von Meyer and Lattermoser (Taylor, '21), in 1897, were the first investigators to recognize that the addition of a very stable sol to a less stable sol, that is, an emulsoid to a suspensoid, prevented the precipitation of the latter by salts. The latter author reached the conclusion that the addition of very stable colloids, such as albumin, gelatin, agar, or gum arabic, to a silver sol, prevented precipitation by electrolytes until the stable colloid is gelatinized. In 1902, Zsigmondy ('09) investigated this "protective" action of colloids quantitatively by means of his gold sol, which in pure aqueous solution is itself very stable, but is also very sensitive to salts. The degrees of protection of various substances, such as gelatin, casein, egg-albumin, dextrin, starches, cane sugar, etc., were expressed by the "gold number."

Further discussion in this paper will be limited to the use of protective colloids in the preparation and utilization of spray materials.

Various organic and inorganic substances have been recommended by different investigators for increasing the spreading and adhesive qualities of fungicides and insecticides. Though many of the early investigations dealt primarily with the spreading and adhesive qualities of sprays, it is reasonable to assume that the physical qualities were also improved in many cases.

Glue was one of the first substances used as a spreader. Mil-
lardet and Davis (Moore, '21) in 1885 used glue with Bordeaux mixture in the treatment of mildew of the vine, thinking that glue increased the efficiency of the mixture. Lowe ('96) found that glue added to lead arsenate, in the proportion of 2 quarts to 45 gallons of spray, gave satisfactory results in spreading the spray evenly over the foliage of cottonwood. Surface ('05) used glue to increase the adherence of lime-sulphur spray.

More recently Jones ('19) has obtained a patent covering the use of glue as a stabilizer for oil emulsions so that they will mix with lime-sulphur solutions. Yothers and Winston ('24) state that such substances as casein, skimmed milk powder, gelatin, corn meal, wheat flour, and starches are as effective as glue in rendering oil emulsions miscible with lime-sulphur solutions.

Various kinds of soaps have been very commonly employed as spreaders. Gillette ('90) was one of the first investigators to report the use of soap as a spreader for insecticides, namely, Paris green and London purple. Washburn ('91), Galloway ('92), and Swingle ('94) are other early investigators to recommend the use of soaps.

A little later those interested in the field began to investigate the use of spreaders quantitatively. Van Slyke and Urner ('04) showed by chemical analysis that different kinds of soaps varied greatly in water content. Therefore, it was concluded that the addition of a definite quantity of soap to sprays may or may not increase their spreading qualities.

Vermorel and Dantony ('10) studied the surface tension of soap solutions in relation to their ability to spread over a surface. They state that whether or not the addition of soap to fungicides will increase their spreading qualities depends upon the method used in the preparation of the mixture. Parker ('11) recommended the addition of soap to certain arsenical sprays to retard settling and likewise increase spreading qualities.

Lovett ('18, '20) made a rather extensive study of the use of spreaders with arsenical sprays. He concluded that the ability of a solution to hold arsenate in suspension, while not necessarily a criterion as to its value as a spreader, does indicate a physical quality in the solution much to be desired in a spreader. Various organic substances were tested, such as caseinate, glue, gelatin,
sage tea, soap bark, and starches. He arranged the materials tested in the order of their merit, based on compatibility, efficiency, availability, cost, and ease of preparation, as follows: caseinate, glue, gelatin, and soap bark. A number of inorganic compounds were also tested. Oil emulsion gave very promising results, while other inorganic materials were unsatisfactory.

Moore ('21)', in a quantitative study of the spreading and adherence of arsenical sprays, found that the addition of materials similar in chemical constitution to the leaf surface causes the spray mixture to form a continuous film over the foliage. Various protein substances and plant infusions gave effective spreading on leaves with surfaces of cellulose, even when they are strongly cutinized. The suspensions containing small-sized particles were found to adhere better than those of larger-sized particles.

Recently, Brinley ('23) has recommended a method of preparing colloidal lead arsenate. The method consists in precipitating the lead arsenate in the presence of a protective colloid, such as gelatin, by the chemical reaction between lead nitrate and disodium arsenate. He found that the method of mixing and diluting the chemicals was a very important factor in obtaining a solution of greatest stability.

Since most of the evidence in the foregoing references points to organic substances as being more effective than inorganic substances in preserving the stability of solutions, it was thought desirable to test the hypo-H$_2$SO$_4$ mixture as prepared with certain glutinous colloids. Thus dextrin, peptone, starch, gelatin, and glue were tested in varying percentages. Dextrin and peptone did not prove of any value in preserving the stability of the solution. While starch possesses certain desirable characteristics, gelatin and glue proved to be more satisfactory. The results obtained with gelatin were slightly less desirable than those obtained with glue. Therefore, considering the materials upon their merits, as to efficiency, cost, and availability, glue should be recommended in preference to gelatin.

The hypo and H$_2$SO$_4$ were diluted in a number of ways before mixing and the stability of the resulting solutions was tested as prepared with glue. Likewise the stability of the solution was
tested as prepared with different proportions of the chemicals. Smaller quantities of the hypo in proportion to the H$_2$SO$_4$ gave best results. The most satisfactory results were obtained by introducing the glue immediately after the chemicals were mixed. If the mixture is allowed to stand any length of time before the glue is added, the stability of the solution is lost; and, further, the introduction of glue to either chemical before mixing results in the loss of a considerable quantity of sulphur, the latter being thrown out of solution in an insoluble mass.

Since temperature has been shown to be an important factor in the preparation of other colloidal solutions, as well as sulphur, the effect of employing warm solutions was tested, with thoroughly satisfactory results. Wackenroder ('46) observed that low temperatures caused a large part of the sulphur particles to settle out of solution.

In order to neutralize the hypo-H$_2$SO$_4$ mixture, while at the same time preserving the stability of the solution and likewise the toxic property of the colloidal sulphur, it was necessary to employ various alkalies. After preliminary tests, strong alkalies were eliminated as unsatisfactory. Sodium carbonate and some other weak alkalies proved satisfactory. Raffo ('08) used Na$_2$CO$_3$ to neutralize a sulphur suspension, made from hypo and H$_2$SO$_4$, after the sulphur had been centrifuged out of suspension and again peptized with water. The greater part of the sulphur was precipitated, leaving a stable solution of sulphur containing a little Na$_2$SO$_4$. The use of glue prevented, to a large extent, the precipitation when weak alkalies were added.

After testing various methods as indicated in the foregoing discussion, the method which proved most satisfactory was as follows: Fifty gms. of hypo were dissolved in 40 cc. of water and warmed to 40–50° C.; 40 cc. of H$_2$SO$_4$, specific gravity 1.84, were measured into a 500-cc. glass cylinder. The warm saturated solution of hypo was added slowly to the H$_2$SO$_4$ with occasional stirring. Eighty cc. of warm water (30–40° C.) were immediately added to the mixture, followed by the same quantity of 1 per cent glue solution of the same temperature. The temperature, as indicated in each case, is a very important factor and one on which, to a large extent, the stability of the solution depends.
The mixture was allowed to stand 48 hours, after which time the reaction was adjusted to $P_H 4.2$ with a saturated solution of $Na_2CO_3$ and then aerated for 30 minutes. The aeration is necessary to free the solution of traces of $SO_2$ which is very injurious to the foliage of plants. The addition of water as indicated is a very important step in the process. It changes the physical state of the solution to such an extent that the solution will remain stable for several days without the addition of a protective colloid. In this connection, Sobrero and Selmi (Taylor, '21), in 1850, investigating colloidal sulphur formed by the reactions of $SO_2$ and $H_2S$ in water, make the following remark: "If water is added to it [colloidal sulphur] it divides up, forming an emulsion from which it does not separate out, even on prolonged standing [several months]."

A saturated solution of dibasic sodium phosphate may be used for neutralizing the mixture. It may be added to the hypo-acid mixture immediately, thereby eliminating the necessity of allowing the mixture to stand 48 hours before adjusting the reaction.

After the two solutions were thoroughly tested on the foliage of plants, toxicity tests were made. The method employed in preparing the sulphur suspensions for testing was essentially the same as that used by Young ('22) in his toxicity studies, and was as follows: Three test-tubes were provided with pipettes that extended through the cork stoppers to the bottom of the tubes. By this means drops could be transferred readily to the hanging-drop cells. Ten cubic-centimeters of the slightly buffered solution, $P_H 4.2$, were added to each tube. This gave a duplicate series, one tube each for the two sulphur suspensions to be tested and one for the control culture. One cubic centimeter of the sulphur suspensions was added to the tubes.

The technique of planting the hanging-drop cultures was the same as that employed by Webb ('21) in his germination studies and later slightly modified by Young ('22), and was as follows: Ground-glass rings were cemented to glass slides by means of parawax and petrolatum. Two of these rings were placed on each slide and 3 slides constituted a series for each organism. A large drop of the sulphur suspension to be tested for toxicity
was placed in the bottom of the two cells. Another drop was placed on each slide and three slides constituted a series for each organism. A large drop of the sulphur suspension to be tested for toxicity was placed in the bottom of the two cells. Another drop was placed on a clean sterile glass slide. A definite spore suspension was made in the drop. By means of a small sterile glass rod a small part of the drop was transferred to a clean sterile cover-glass and spread out in the form of a smear. The cover-glass was inverted and sealed air-tight by means of petrolatum over the glass cell. The cultures were incubated at 23° C. and germination counts made at the end of 18 hours. The results are recorded in table I.

TABLE I

TOXICITY OF CERTAIN FORMS OF COLLOIDAL SULPHUR. THE FIGURES INDICATE PERCENTAGE OF GERMINATION.

<table>
<thead>
<tr>
<th>Form of sulphur</th>
<th>Botrytis cinerea</th>
<th>Colletotrichum Gossypii</th>
<th>Glomerella cingulata</th>
<th>Macrosorum sarcinaeforme</th>
<th>Sclerotinia cinerea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control—without sulphur</td>
<td>80</td>
<td>65</td>
<td>76</td>
<td>94</td>
<td>60</td>
</tr>
<tr>
<td>Colloidal sulphur neutralized with Na₂CO₃</td>
<td>5</td>
<td>0</td>
<td>8</td>
<td>44</td>
<td>2</td>
</tr>
<tr>
<td>Colloidal sulphur neutralized with Na₂HPO₄</td>
<td>15</td>
<td>4</td>
<td>12</td>
<td>57</td>
<td>5</td>
</tr>
</tbody>
</table>

1 Average of duplicate tests run at two different times.

The results in this table show that both colloidal sulphur solutions tested were toxic to all the organisms used. The solution prepared with disodium phosphate was slightly less toxic than that prepared with Na₂CO₃. This can be accounted for in that it required more of the phosphate to neutralize the mixture. Therefore, there was less sulphur in the mixture.
THE PREPARATION OF COLLOIDAL SULPHUR FROM LIME SULPHUR

Since the introduction of lime sulphur as an orchard spray serious injuries to foliage of plants have been frequently reported as a result of its use. Injury is often more frequent when the spray is used in combination with certain arsenicals. This is shown by a survey of the literature from various agricultural experiment stations. A number of methods for the elimination of these difficulties have been suggested by different investigators, all of which relate primarily to the method of diluting, mixing, and applying the spray, or to the time of application, or the evidence of inferior compounds. Since lime sulphur is composed largely of sulphides (pentasulphides) the caustic action on foliage of plants has been attributed to these soluble compounds. A few investigators have suggested that the injurious properties can be eliminated by precipitating the soluble sulphides with salts or acids. These methods, however, have not come into general use.

Wallace ('10), in a study of lime-sulphur spray injury, precipitated commercial lime sulphur, diluted 1-30 with water, by applying it with a carbonic-acid gas sprayer. The solution was allowed to stand in contact with the gas one-half hour before the application of the spray. A heavy white precipitate was formed, and this did not cause injury to apple and peach foliage when used alone as a spray. However, when lead arsenate was introduced into the mixture before the precipitation, the resulting material used on young peach trees caused almost complete defoliation.

Stewart ('12), in a similar study, pointed out that lime-sulphur-arsenical injury could be eliminated by precipitating the mixture with iron sulphate, or "copperas," added at the rate of 3½ pounds to 50 gallons of 1.01 spray solution. The mixture was found to be very disagreeable to handle, due to its tenacious and blackening qualities. Also serious discoloration of fruit resulted from its use, and the effectiveness of arsenicals was reduced. Waite ('10) had previously reported the use of iron sulphate and copper sulphate in the preparation of self-boiled lime sulphur.

Safro ('13), after a study of lime sulphur spray injury, con-
cluded that the effect was due to the soluble sulphide content of the spray. He found that the injury could be reduced by rendering the sulphides insoluble, that is, by precipitating them. His results indicated that the injury could be reduced by the addition of either iron, copper, or zinc sulphate, the salt being first dissolved in water, in the proportion of 4 pounds to 100 gallons of diluted (1 to 20) lime-sulphur solution. Carbon dioxide and H2SO4 were also used, the substances being introduced until the remaining sulphides gave the spray an amber color. The carbon dioxide entirely eliminated the injury.

More recently, definite methods have been given for precipitating lime sulphur for commercial use. Ramsay and Cooke ('22) describe a method of preparing colloidal sulphur from lime sulphur which has been used effectively as a spray in Australia. The method recommended is as follows: Ten gallons of home-made lime sulphur, 26° Baumé, were diluted with 25 gallons of water in a barrel of 40 or 50 gallons capacity. Using a porcelain or earthenware vessel, 6 pints of strong commercial H2SO4 were poured slowly into 9 pints of cold water and the solution allowed to cool. The cold diluted H2SO4 was then added to the diluted lime sulphur, 2 or 3 ounces at a time, stirring well after each addition of acid, until the typical yellow color of the original lime-sulphur disappeared and until the addition of H2SO4 produced no further precipitation of sulphur. The precipitated sulphur was allowed to settle for a day or two and the clear liquid above was siphoned or decanted off. Three pounds of cheap glue were dissolved in just sufficient hot water to render the glue soluble, and, while still hot, stirred thoroughly into the precipitated sulphur. For use, the mixture thus obtained was diluted with water to 250 gallons. This gave approximately 5 pounds precipitated sulphur per 100 gallons of spray.

After making a study of the toxicity of the various ingredients of decomposed lime sulphur, Young ('22) showed conclusively that the lasting toxicity was due to the precipitated sulphur. This form of precipitated sulphur was found to be about as toxic to parasitic fungi as "hydrophobic" colloidal sulphur. Later ('25) he prepared colloidal sulphur from lime sulphur as a summer spray. The results from the use of this material in
combating apple scab were very encouraging. His method was as follows: Ten gallons of lime sulphur, 32° Baumé, were placed in a 50-gallon barrel. To this were added 10 gallons of water and 1 pound of glue, the latter previously dissolved in hot water. Sulphuric acid diluted with 3 parts of water was then added until the reaction of the mixture was \( \text{pH} 4.2 \). The mixture was allowed to settle and the supernatant liquid decanted off. The mixture contained about 1 pound of active sulphur per gallon. Five gallons of the precipitate were diluted with water to 95 gallons before using as a spray.

Since the recent uses of precipitated lime sulphur as a spray have given encouraging results, it was considered important to make further studies upon its preparation with the object of producing a mixture of greater stability. Likewise from previous tests of toxicity (Young, '22), the mixture would be expected to exhibit greater fungicidal properties. There were several factors which were thought to be important in the preparation, namely: (1) the choice of acids and the dilution of these; (2) the concentration and method of diluting the lime-sulphur solution; (3) the temperature; and (4) the protective colloid, its concentration, and the method of introduction.

The lime sulphur used for this work was a freshly made solution, prepared according to the Van Slyke method, that is, in the proportion of 80 pounds sulphur, 36 pounds lime (CaO), and 50 gallons of water, these ingredients being boiled together one hour. Preliminary tests were made with a number of acids and phosphates to determine which of those were most desirable for precipitating the freshly made lime sulphur. The use of phosphates, of certain acids, and also of strong solutions of acids gave unsatisfactory results and these procedures were eliminated. Weak solutions of \( \text{HNO}_3 \) and \( \text{H}_2\text{SO}_4 \) gave very promising results. Glue, which proved to be the most satisfactory in the previous preparations, was used as a protective colloid. The concentration of the glue, the method of introducing it, the dilution of the lime sulphur, and the temperature of the solutions employed in the preparation were found to be the important factors upon which, to a large extent, the stability of the solution depended.

Since the preliminary tests showed that the dilution of the
acid used in precipitating lime sulphur had an important bearing upon the stability of the solution, further tests were made in order to determine what strength of HNO₃ and H₂SO₄ could be most satisfactorily employed. Twenty-five cc. of lime-sulphur solution were measured into each of 10 glass beakers of 300 cc. capacity. The beakers were numbered 1-5 inclusive, which gave a duplicate set, one set each for the two acids to be tested. The mixtures were then adjusted to the reaction pH 4.2, with dilutions of HNO₃ and H₂SO₄ as follows:

No. 1. Acids diluted 1-3 with water.
No. 2. Acids diluted 1-5 with water.
No. 3. Acids diluted 1-10 with water.
No. 4. Acids diluted 1-20 with water.
No. 5. Acids diluted 1-40 with water.

The resulting solutions showed a striking range in stability. The precipitated sulphur in solutions No. 1 settled out of suspension within a few hours after it was prepared. Solutions No. 2 remained stable several days. Solutions No. 3 remained stable about 2 weeks. Solutions No. 4 remained stable several months. Solutions No. 5 were less stable than No. 3. The solutions prepared with HNO₃ possessed slightly greater stability in all cases than those prepared with H₂SO₄. These results show conclusively that the strength of the acid used in precipitating the sulphur is an important factor in producing a product of the desired stability.

After determining the proper dilution of acid to use, further studies were made of different procedures in making the preparation. The method which proved the most satisfactory is as follows: Fifty cc. of freshly made lime sulphur were measured into a glass beaker of 600 cc. capacity. One gram of dry flake glue was dissolved in 200 cc. of water and mixed with the lime sulphur while warm (35-40° C.). Ten cc. of HNO₃ were diluted with water in a separate container. The diluted HNO₃ was then added to the diluted lime-sulphur mixture very slowly, with stirring, until the reaction pH 4.2 was obtained. The mixture was allowed to set for several hours in the open container, with occasional stirring, to remove the H₂S. This gave a final dilution, in respect to the original lime-sulphur solution, of 1 to 9.
The mixture contained approximately 1.8 per cent sulphur. Colloidal sulphur prepared by this method will remain stable for several months. Sulphuric acid diluted 1-20 may be used to precipitate the lime sulphur mixture. However, as previously indicated, the solution as prepared with H₂SO₄ is less stable.

In determining the toxicity of this form of colloidal sulphur, the mixtures prepared with HNO₃ and H₂SO₄ were compared with lime sulphur precipitated by the CO₂ in the air, the latter being effected as follows: Ten cc. of lime sulphur solution were diluted with 60 cc. of water and this solution was poured into a large open dish and allowed to decompose. The precipitate was then taken up in 70 cc. of water. One cc. of each of these 3 precipitated lime-sulphur solutions was then added to 5 cc. of the slightly buffered mannite-H₃PO₄-NaOH mixture of pH 4.2 (except in tests with Macrosporium sarcinaeforme, where M/5 mannite was used), and the toxicity determined as before. The organisms used and the results are recorded in table II.

| TABLE II |
| TOXICITY OF PRECIPITATED LIME-SULPHUR SOLUTIONS. THE FIGURES INDICATE THE PERCENTAGE OF GERMINATION (AVERAGE OF CULTURES RUN AT 2 DIFFERENT TIMES) |

<table>
<thead>
<tr>
<th>Form of sulphur</th>
<th>Botrytis cinerea</th>
<th>Glomerella cingulata</th>
<th>Macrosporium sarcinaeforme</th>
<th>Sclerotinia cinerea</th>
<th>Ustilago Hordei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control—without sulphur</td>
<td>84</td>
<td>64</td>
<td>88</td>
<td>59</td>
<td>73</td>
</tr>
<tr>
<td>Lime sulphur solution precipitated with HNO₃</td>
<td>24</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Lime sulphur solution precipitated with H₂SO₄</td>
<td>55</td>
<td>13</td>
<td>30</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Lime sulphur solution precipitated by CO₂ in air</td>
<td>71</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>21</td>
</tr>
</tbody>
</table>

From the results exhibited in table II it is clear that the toxicity of the sulphur prepared by the different methods is related to...
the stability of the sulphur suspensions. The sulphur precipitated from lime sulphur by the CO₂ in the air settles out of suspension in a very short time, and is therefore the least toxic of the three forms of sulphur tested. The solution prepared with HNO₃ possesses the greatest stability and likewise exhibits the greatest toxicity.

The mixtures prepared with HNO₃ and H₂SO₄ were thoroughly tested on the foliage of plants already named growing in the greenhouse. They were found to spread over the surface of the foliage evenly and adhere well; in fact it was difficult to remove the films by washing with a strong stream of water from the hose.

PREPARATION OF COLLOIDAL SULPHUR BY MEANS OF SO₂ AND H₂S

The formation of colloidal sulphur by the reaction between SO₂ and H₂S in water was first recorded by Berthollet (1798). The reaction was later studied by Wackenroder ('46) who discovered pentathionic acid and prepared it by passing H₂S into a saturated solution of SO₂, always keeping an excess of the latter. Lewes ('81), Spring ('82), Shaw ('83), and Débus ('88) are other early contributors to the study of the properties of pentathionic acid formed by this process. The reaction of SO₂ and H₂S has been studied more recently by Riesenfeld and Feld ('21) in a study of polythionic acid and polythionate.

Selmi and Sobrero ('50) (also earlier investigators) made rather extensive studies of colloidal sulphur formed by the reaction of SO₂ and H₂S. A review of the work is given by Odén ('13). That there is more than one state of colloidal sulphur formed by this process is shown by Selmi ('52) who prepared colloidal sulphur as follows: Sulphur dioxide was passed into distilled water until a saturated solution was formed. Hydrogen sulphide was then passed into the saturated solution of SO₂, care being taken not to have an excess of H₂S, as it precipitates the soluble sulphur. The solution was then centrifuged to remove the larger particles and the supernatant liquid coagulated with sodium chloride. The coagulum was then peptized in water. Further evidence of the two forms of colloidal sulphur is given in the papers cited above. Young ('22) has designated the two forms, according to their degree of hydration, as hydrophilic
and hydrophobic colloidal sulphur. There are in all probability several grades of fineness in the hydrophobic forms. This is shown in the work of Odén ('13).

The colloidal sulphur prepared from \( \text{SO}_2 \) and \( \text{H}_2\text{S} \) in this investigation consists largely of the hydrophobic form. The method used was as follows: A rubber stopper having two holes was provided with two thistle tubes, over the mouths of which finely perforated parchment paper was securely fastened. The tubes were inverted into a 2-liter flask containing 1500 cc. of water, the stopper closing the mouth of the flask. Sulphur dioxide (compressed liquid) and \( \text{H}_2\text{S} \) (made from \( \text{FeS} \) and 1 to 1 muriatic acid) were passed simultaneously into the water through the thistle tubes, keeping the sulphur dioxide slightly in excess. After three hours a slight excess of \( \text{H}_2\text{S} \) was introduced to free the solution of \( \text{SO}_2 \). The suspension thus formed contained 12 per cent sulphur, which had great stability. In fact, there was but very little settling out after standing three months.

The efficiency of the method is determined by the fineness of the perforations through which the gases are introduced into the liquid. In the method described the gases are introduced in the form of a fine spray, which permits a more thorough mixing in the solution. The introduction of the gases in the form of large bubbles was found to be inefficient and unsatisfactory in preliminary tests. The use of distilled water has been found more desirable than tap water, especially since the latter may contain sufficient salts or alkalies to effect a partial precipitation of the colloidal sulphur as it is formed.

The colloidal sulphur solution thus prepared has an acid reaction slightly beyond the acid range of available indicators, \( \text{pH} \) 1.0. Wackenroder ('46) found that some \( \text{H}_2\text{SO}_4 \) was formed during the process in the presence of oxygen. It was later found difficult to free the mixture entirely of \( \text{SO}_2 \), and it was evident that traces were left in the solution. The solution was found to be injurious to the foliage of plants; therefore, it was necessary to adjust the reaction, or treat the solution in some way before it could be used as a spray.

The weak alkalies used for adjusting the colloidal sulphur
solution prepared by the hypo-H$_2$SO$_4$ method, namely, Na$_2$CO$_3$ and the Na$_2$HPO$_4$, caused precipitation of the colloidal sulphur when freshly prepared by this method. Similar results were also produced by the introduction of glue or gelatin. However, promising results were obtained by aerating the mixture for 48 hours and then adjusting the reaction to pH 4.2 with a weak solution of Na$_2$HPO$_4$. The resulting solution remained stable for 3 months, after which time the colloidal sulphur was precipitated. This material was found to be very toxic to fungi when first prepared. It proved effective for control of carnation rust in the greenhouse.

Later the colloidal sulphur was coagulated with NaCl and allowed to settle out of solution. The method was as follows: A saturated solution of NaCl was added to the freshly prepared colloidal sulphur suspension until it changed from a bright to a pale yellow color. The mixture was allowed to set two hours, after which time the coagulum had settled out of suspension. The supernatant liquid was then siphoned off and the coagulum washed with water and allowed to settle again. The supernatant liquid was again siphoned off. The sulphur is left in the form of a thin paste, containing about 70 per cent water and a little NaCl. The sulphur readily assumes the colloidal state when further quantities of water are added. Selmi and Sobrero ('50) found that colloidal sulphur prepared from SO$_2$ and H$_2$S could be coagulated by a neutral sodium salt, and again be emulsified in water. They state that when potassium salts are used the precipitated sulphur completely loses the property of emulsifying in water.

When the sulphur paste (prepared by the above method) is resuspended in water, shortly after it has been coagulated, the suspension remains stable over a long period of time. In fact, such a suspension was kept in the laboratory for 8 months, and after this there was still little or no settling of the sulphur. However, if the coagulum is not taken up in water, the sulphur begins to crystallize in about 1 month and soon loses its colloidal property. The paste form would be much more desirable for practical use. Therefore further investigations should be made to determine if it can be treated in such way as to prevent crystallization.
The results on the toxicity tests of this form of colloidal sulphur are discussed on subsequent pages.

THE TOXICITY OF CERTAIN FORMS OF COLLOIDAL SULPHUR IN RELATION TO THE HYDROGEN-ION CONCENTRATION

Since it was shown by Young ('22) that the hydrogen-ion concentration has a marked influence upon the toxicity of certain forms of colloidal sulphur, it was thought important to determine if this influence may be exhibited by other forms of these colloidal preparations. A colloidal sulphur solution was prepared from SO₂ and H₂S as already described, with modifications as follows: After the colloidal sulphur had been coagulated with NaCl, the solution was then centrifuged for 15 minutes at 1500 revolutions per minute. The supernatant liquid was then decanted. Upon drying and weighing, the coagulum was found to contain 50 per cent of sulphur.

One gm. of the coagulum was weighed out and taken up in 100 cc. of distilled water. This gave a dilution of sulphur of 0.5 gm. in 100 cc., or 1 to 200. When freshly made, the reaction of the solution was P₉ 4.0; after standing in a closed container for 1 week the P₉ had changed to 2.2. The freshly made sulphur suspension also changed the hydrogen-ion concentrations of the culture solutions. Therefore it was found necessary to adjust the sulphur suspensions to the range of hydrogen-ion concentrations corresponding to the particular hydrogen-ion concentration of the culture solution, which was done as follows: Fifty-cc. quantities of the 1 to 200 colloidal sulphur suspension of P₉ 4.0 were measured into each of 11 clean glass beakers. The suspensions were then adjusted toward and into the alkaline range with an increasing number of drops of M/10 Na₂HPO₄ solution, and into the acid range with M/10 H₃PO₄ solution. This gave a range of hydrogen-ion concentrations as follows: P₉ 2.4, 3.4, 4.2, 5.0, 5.4, 5.8, 6.2, 6.8, 7.4, and 8.4. The colloidal sulphur suspension thus prepared did not change the hydrogen-ion concentrations of the culture solution during the 18-hour period of incubation of the cultures. The stability of the sulphur suspensions of different hydrogen-ion concentrations will be discussed later.
In later work it was found that if the coagulation, centrifuging, and peptizing with water is repeated 3 times, the hydrogen-ion concentration of the resulting colloidal sulphur suspension does not change even on prolonged standing. Likewise, such a suspension does not evert the hydrogen-ion concentration of the culture solution. This is probably due to the buffer action of the phosphate which was added.

The culture solution used for making the toxicity studies was the slightly buffered mannite-$\text{H}_3\text{PO}_4$-$\text{NaOH}$ mixture prepared according to the method already described. Twenty test-tubes were provided with pipettes that extended through the cork stopper to the bottom of the tubes, according to the method employed by Young ('22). This constituted a duplicate series of 10 each, and 10 cc. each of the slightly buffered solutions were added to the tubes so that each tube represented a particular
hydrogen-ion concentration. The colloidal sulphur suspensions were added to one series, with accurately calibrated pipettes, in quantities to make the dilutions indicated in table III.

The hanging-drop cultures were prepared in closed-ring cells according to the method already described, and were incubated at 23° C. After germination of 18 hours, counts were made. The results of these tests are shown in table III, also in figs. 1-4.

![Graph showing germination percentage against pH]

Fig. 2. Germination of spores of *Gloeosporium venetum* in hanging-drop cultures: toxic action of colloidal sulphur prepared by method 1 — ---; control, without sulphur ———.

The results show that the concentration of the sulphur employed in the culture solutions was a very important factor. The organisms tested vary greatly in their resistance to the toxicity of this form of colloidal sulphur, *Macrosporium sarcinaeforme* being very resistant, while extremely weak dilutions are sufficient to kill *Sclerotinia cinerea*. In general, this form of sulphur is most toxic toward, and in, the alkaline range of hydrogen-ion concentrations. The highest percentage of germination was in most cases at $P_n$ 3.4. Beyond this point toward the
alkaline range there was a gradual decrease to the point $p_H$ 5.8, after which there is a sharp decrease.

The influence of the hydrogen-ion concentration upon the toxicity of this form of colloidal sulphur differs from the results obtained by Young ('22) with his hydrophilic colloidal sulphur. In order to make a comparison by the same method, hydrophilic colloidal sulphur was prepared according to the method of Young ('22) which was as follows: Fifty gms. of pure crystalline sodium thiosulphate were dissolved in 30 cc. of distilled water; 70 gms. of concentrated $H_2SO_4$, sp. gr. 1.84, arsenic-free, were weighed into a glass cylinder of 300 cc. capacity. The cylinder was placed in a vessel of cold water, and the saturated solution of sodium thiosulphate added very slowly with occasional stirring. The mixture was then allowed to cool, and 30 cc. of distilled water were added. The preparation was then placed in a water bath and warmed to 80° C. for 10 minutes and filtered through glass.
wool to remove insoluble sulphur. After the mixture was allowed to cool, concentrated NaCl was added and the mixture centrifuged for 30 minutes at 1500 revolutions per minute. The coagulum was then peptized with 100 cc. of distilled water and the insoluble sulphur centrifuged out. The peptized sulphur was then treated 3 times with 25 cc. of saturated NaCl and finally peptized by adding 1 gm. of the coagulum to 100 cc. distilled water. The solution had a $P_H$ of 4.2, which does not change upon standing.

![Graph of Germination]

**Fig. 4.** Germination of spores of *Ustilago Hordei* in hanging-drop cultures: toxic action of certain forms of colloidal sulphur; prepared by method 1 — — — —; prepared by method 1, crystallized — — — —; prepared by method 2 — — — — — —; control, without sulphur — — — — — —

By means of an accurately calibrated pipette, aliquot parts were measured into each of 10 tubes containing the slightly buffered solution, to make the dilutions indicated in table III. The hanging-drop cultures were made in exactly the same way as described for the other forms of colloidal sulphur, and germination counts were made after an incubation interval of 18 hours at 23° C. The results are shown in table III and in figs. 1, 3, and 4.
### Table III

**Toxicity of Certain Sulphur Suspensions. The Figures Represent Percentage of Germination (Average of 2-5 Tests)**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Form of colloidal sulphur</th>
<th>Hydrogen-ion concentration (pH)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dilution of sulphur</td>
<td>2.4</td>
</tr>
<tr>
<td>Botrytis Allii</td>
<td>Control, without sulphur</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Prepared by Method 1</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Prepared by Method 2</td>
<td>0</td>
</tr>
<tr>
<td>Botrytis cinerea</td>
<td>Control, without sulphur</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>Prepared by Method 1</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Prepared by Method 2</td>
<td>1</td>
</tr>
<tr>
<td>Colletotrichum Gossypii</td>
<td>Control, without sulphur</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Prepared by Method 1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Control, without sulphur</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Prepared by Method 1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Prepared by Method 2</td>
<td>0</td>
</tr>
<tr>
<td>Gloeosporium venetum</td>
<td>Control, without sulphur</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Prepared by Method 1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Prepared by Method 2</td>
<td>0</td>
</tr>
<tr>
<td>Organism</td>
<td>Form of colloidal sulphur</td>
<td>Dilution of sulphur</td>
</tr>
<tr>
<td>------------------</td>
<td>---------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.4</td>
</tr>
<tr>
<td>Macrosporum sarcinaeforme</td>
<td>Control, without sulphur</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Prepared by Method 1</td>
<td>1</td>
</tr>
<tr>
<td>Sclerotinia cinerea</td>
<td>Control, without sulphur</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>Prepared by Method 1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Prepared by Method 2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Control, without sulphur</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Prepared by Method 1</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>Prepared by Method 2</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Prepared by Method 1, sulphur crystallized</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Prepared by Method 2</td>
<td>22</td>
</tr>
</tbody>
</table>
In the table and figures just referred to, the form of colloidal sulphur is indicated according to the method of its preparation. Thus, Method I refers to the colloidal sulphur prepared by the \( \text{SO}_2-\text{H}_2\text{S} \) process, and Method 2 refers to the hydrophilic form of colloidal sulphur prepared with sodium thiosulphate and \( \text{H}_2\text{SO}_4 \).

The results of the toxicity tests of the two forms of colloidal sulphur show that Young's hydrophilic form is much more toxic to the organisms used, as it required much smaller quantities of this form to inhibit germination of spores. This toxicity corresponds to the size of the sulphur particles. It has already been indicated that the form prepared by the \( \text{SO}_2-\text{H}_2\text{S} \) method is largely the hydrophobic form with probably a small amount of the hydrophilic form.

The influence of the hydrogen-ion concentration upon the hydrophilic colloidal sulphur was found to be essentially the same as that recorded by Young ('22). Upon examining the culture tubes containing the hydrophilic colloidal sulphur, it was also found that settling out was rapidly increased as the \( \text{pH} \) increased beyond \( \text{pH} 5.4 \). However, this does not explain the difference in toxicity of the two forms. It appears logical that if the toxic substance, exhibited by the colloidal sulphur prepared by the \( \text{SO}_2-\text{H}_2\text{S} \) method, is pentathionic acid ("truly 'soluble' sulphur") it would be precipitated at a higher \( \text{pH} \) than 5.4, as has been shown to be the case with hydrophilic colloidal sulphur. Further proof that pentathionic acid would be precipitated, particularly in the more alkaline solution, is shown by the investigations of Wackenroder ('46), Shaw ('83), Débus ('88), and others. Therefore, it is shown conclusively that the toxic substance exhibited by the form of colloidal sulphur prepared from \( \text{SO}_2 \) and \( \text{H}_2\text{S} \) possesses properties different from the toxic substance exhibited by the hydrophilic form, prepared according to the method of Young. From the evidence, the latter is, in all probability, pentathionic acid.

There were some indications that the toxic substance exhibited by the colloidal sulphur prepared from \( \text{SO}_2 \) and \( \text{H}_2\text{S} \) may be destroyed by repeating the coagulation, centrifuging, etc., several times. This remains to be established by more detailed study.
It was later found that when colloidal sulphur was prepared by the $\text{SO}_2$-$\text{H}_2\text{S}$ method, coagulated with $\text{NaCl}$, and kept for some time in coagulated form, the sulphur soon crystallized and would no longer remain in suspension when water was added. Suspensions were made of this crystallized sulphur and tests were made in the usual way with *Botrytis cinerea* and *Ustilago Hordei*. The results of these tests, in table III, show that the toxic substance has been destroyed to a large extent.

**THE INFLUENCE OF THE HYDROGEN-ION CONCENTRATION UPON THE STABILITY OF COLLOIDAL SULPHUR SUSPENSIONS, AND THE RESULTING EFFECT UPON THE TOXICITY**

Since it was shown in the toxicity tests that the form of colloidal sulphur prepared by the reaction of $\text{SO}_2$ and $\text{H}_2\text{S}$ exhibits its greatest toxicity at a higher $\text{P}_\text{H}$ than 4.2, it was considered important to determine whether the hydrogen-ion concentration has any influence upon the stability of the suspension. A colloidal sulphur dispersion was prepared by the $\text{SO}_2$-$\text{H}_2\text{S}$ method, coagulated with $\text{NaCl}$ and centrifuged. The coagulum was then weighed and taken up with distilled water, in the proportion of 1 gm. of sulphur to 200 cc. of water. Fifty-cc. portions of the suspension were measured into each of 11 beakers. The $\text{P}_\text{H}$ of the suspension was 4.2. The suspensions were then adjusted towards, and into, the alkaline range with M/10 $\text{Na}_2\text{HPO}_4$, and on the acid side with M/10 $\text{H}_2\text{PO}_4$. The resulting preparations had the following range of hydrogen-ion concentrations: $\text{P}_\text{H}$ beyond the acid range, 2.4, 3.2, 4.2, 5.0, 5.4, 5.8, 6.2, 6.8, 7.4, and 8.4. The acid solution was adjusted slightly beyond the acid range of available indicators. Portions of these solutions were transferred to clean test-tubes, and the tubes were closed with corks. The remaining portions were used in the subsequent experiment.

The suspensions showed a very striking range in stability. Settling out of the sulphur was increased rapidly as the $\text{P}_\text{H}$ increased beyond 5.8. A photograph of the tubes was made after 8 months and is shown in pl. 20. The stability of the preparations was as follows: $\text{P}_\text{H}$ 8.4 began to aggregate and settle in about 1 week and had settled out completely in 2 weeks. $\text{P}_\text{H}$ 7.4
settled out in 3 weeks. \( \text{P}_H \) 6.8 settled out in 4 weeks. \( \text{P}_H \) 6.2 had settled out to a large extent after 7 weeks. \( \text{P}_H \) 5.8 changed from a bright yellow to cream-white in about 8 weeks, with some settling out. After 8 months this solution had largely settled, but the photograph does not bring this out clearly because of sulphur particles sticking to the sides of the tubes, which could not be removed without agitation of the solution. The acid solution (that is, the solution which was adjusted beyond the acid range of available indicators) began to settle in about 1 month and continued slowly. There was still some sulphur in the suspension after 8 months, as shown in pl. 20. No great difference was manifest in the stability of the preparations represented by \( \text{P}_H \) 2.4, 3.2, 4.2, 5.0, and 5.4. There was some sediment in all the tubes after 8 months, but the range from \( \text{P}_H \) 4.2 to 5.4 shows greatest stability.

The tubes were shaken, and 0.5 cc. were taken from each tube and transferred to tubes containing 10 cc. of the slightly buffered solution of like hydrogen-ion concentration. This gave a final concentration of sulphur of 1 to 4,000 which is weaker than that used for other toxicity tests. Spore suspensions of *Botrytis cinerea* were made from the sulphur suspensions, and germination tests were made in the usual way in closed-ring cells. The results are shown in table IV.

**TABLE IV**

**TOXICITY TESTS WITH CERTAIN FORMS OF COLLOIDAL SULPHUR. THE FIGURES INDICATE PERCENTAGE GERMINATION**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Form of sulphur</th>
<th>( \text{P}_H )</th>
<th>2.4</th>
<th>3.2</th>
<th>4.2</th>
<th>5.4</th>
<th>5.8</th>
<th>6.2</th>
<th>6.8</th>
<th>7.4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control, without sulphur</td>
<td></td>
<td>72</td>
<td>83</td>
<td>80</td>
<td>75</td>
<td>57</td>
<td>47</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td><em>Botrytis cinerea</em></td>
<td>Colloidal sulphur adjusted to ( \text{P}_H ) range indicated after standing 8 months</td>
<td></td>
<td>24</td>
<td>60</td>
<td>67</td>
<td>47</td>
<td>62</td>
<td>39</td>
<td>29</td>
<td>10</td>
</tr>
</tbody>
</table>

The results of this test show that when the stability of the sulphur is destroyed by a higher \( \text{P}_H \) than 5.4, the toxic substance is likewise destroyed. In this case there was still some sulphur...
in suspension at $P_H$ 5.8. However, it did not exhibit any toxicity to *Botrytis cinerea*. The suspensions referred to as $P_H$ 2.4, 3.2, 4.2, and 5.4 are all toxic even with the very weak concentration of sulphur used.

THE TOXICITY OF VOLATILE PRODUCTS OF COLLOIDAL SULPHUR

Since it was demonstrated by Young ('22) that hydrophilic colloidal sulphur exhibited the usual degree of toxicity when the spores were not in direct contact with the sulphur, it was thought important to determine whether the toxic substance of colloidal sulphur made by the $SO_2-H_2S$ method would be liberated to the same extent. To test this point, portions of the freshly prepared sulphur suspensions, employed in the preceding experiment, were used. These suspensions had been adjusted, as indicated, to the hydrogen-ion concentration range $P_H$ 2.4–8.4.

The organisms used were *Botrytis Aliii* and *Ustilago Hordei*. The hanging-drop cultures were made in the usual way, with modifications as follows: A small quantity (one-tenth cc.) of each sulphur suspension was placed in the bottom of each cell. The spores were suspended in the slightly buffered mixture without sulphur. In this way the spores were not in direct contact with the sulphur particles, being separated by the height of the cell, which was 8 mm. The cultures were incubated at 23° C. and germination counts made at the end of 18 hours. The results are given in table V.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Form of sulphur</th>
<th>Hydrogen-ion concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2.4</td>
</tr>
<tr>
<td>Botrytis Aliii</td>
<td>Control, without sulphur</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Colloidal sulphur in bottom of cells</td>
<td>2</td>
</tr>
<tr>
<td>Ustilago Hordei</td>
<td>Control, without sulphur</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>Colloidal sulphur in bottom of cells</td>
<td>3</td>
</tr>
</tbody>
</table>
The results in this table are similar to those recorded in table III. The colloidal sulphur exhibits the usual degree of toxicity, that is, the toxicity was greatest beyond $P_H$ 4.2, with freshly prepared suspensions.

THE EFFECT OF DRYING AND AERATING UPON THE TOXICITY OF COLLOIDAL SULPHUR

Since some growers prefer to use sulphur dusts, rather than spray solutions, a test was made to determine whether colloidal sulphur could be dried without destroying its stability and toxic property. A colloidal sulphur suspension was prepared by the $SO_2-H_2S$ method, was coagulated with $NaCl$, and centrifuged. One gm. of the coagulum was weighed on to a clean watch-glass, spread over the surface, and allowed to dry thoroughly at room temperature. After 5 days the sulphur was in the form of a very fine powder. The dry sulphur was taken up with 200 cc. of distilled water. The sulphur assumed the colloidal state readily and the resulting suspension had a reaction of $P_H$ 5.0. Toxicity tests were made in the usual way, and the results are recorded in table vi.

Two series of wash bottles were arranged to test the effect of aeration upon the stability and toxicity of colloidal sulphur. Freshly made colloidal sulphur preparations containing 1 gm. of sulphur in 200 cc. of water and having a reaction of $P_H$ 4.0 were transferred to the wash bottles. Series No. 1 was aerated continuously for 5 days. Air from which the oxygen was removed with alkaline pyrogallol was passed through the duplicate series No. 2.

After 5 days the reaction of each sulphur suspension was tested and found to be $P_H$ 1.4 in Series No. 1, and $P_H$ 1.8 in Series No. 2. The hydrogen-ion concentrations were adjusted to $P_H$ 5.0 with $M/10 Na_2HPO_4$, and toxicity tests were made in closed-ring cells in the usual way. For a comparison a freshly made solution of colloidal sulphur was also tested. Spores of *Sclerotinia cinerea* and *Botrytis Alii* were used as indicators of the toxicity. The results are recorded in table vi. In the table the dilution of sulphur used was 1–20,000 for *Sclerotinia cinerea* and 1–2,000 for *Botrytis Alii*.

1 One part pyrogallol, 5 parts NaOH, and 30 parts $H_2O$. 
TABLE VI
TOXICITY OF PRE-DRIED AND OF AERATED SULPHUR SUSPENSIONS. THE FIGURES REPRESENT PERCENTAGE OF GERMINATION (AVERAGE OF DUPLICATE CULTURES MADE AT DIFFERENT TIMES)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Treatment of sulphur</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control, without sulphur</td>
</tr>
<tr>
<td>Botrytis Allii</td>
<td>71</td>
</tr>
<tr>
<td>Sclerotinia cinerea</td>
<td>57</td>
</tr>
</tbody>
</table>

The results show that drying and continuous aeration for 5 days has no appreciable effect upon the toxicity of this form of colloidal sulphur. Further tests will be required to determine whether there is any influence from more protracted periods of drying and aerating. Likewise, further investigations are necessary to determine what acid or acids are responsible for changing the $P_H$ of the colloidal sulphur solution during the aeration. It was found that the aeration of sulphur "flour" resulted in a change in reaction toward the acid range within a very short time.

THE INFLUENCE OF TEMPERATURE UPON THE TOXICITY OF COLLOIDAL SULPHUR

It has been shown by Doran ('22) that the fungicidal value of sulphur increases with the rise of temperature. Butler ('23) found that the temperature was an important factor in controlling snapdragon rust by the use of sulphur dust. Below 15° C. sulphur was found to be ineffective.

An experiment was arranged to test the influence of a restricted temperature range upon the toxicity of a colloidal sulphur preparation. A suspension containing 1 gm. of colloidal sulphur ($SO_2$-$H_2S$ form) in 200 cc. of water was freshly prepared. The reaction of this suspension was $P_H$ 4.0. A dilution of 1 to 8,000 was made from this suspension in the slightly buffered solution $P_H$ 4.2. This concentration of sulphur was known not to inhibit germination of spores at ordinary temperature. Toxicity tests were made with spores of Botrytis cinerea at the temperatures 18°, 22°, 24°, and 28° C. Controls without sulphur were also
run at the same time. These tests were made in duplicate and conducted at two different times. The results are shown in table VII.

**TABLE VII**

**THE EFFECT OF TEMPERATURE UPON THE TOXICITY OF A COLLOIDAL SULPHUR SUSPENSION. THE FIGURES REPRESENT PERCENTAGE OF GERMINATION**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Form of sulphur</th>
<th>Temperature °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>Botrytis cinerea</td>
<td>Control</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Colloidal sulphur</td>
<td>18</td>
</tr>
</tbody>
</table>

Toxicity was exhibited at the lowest temperature tested, though the higher temperatures increased the toxicity to a considerable extent. It would be of importance to test the effect of a wider range of temperatures and with a variety of organisms.

**PRACTICAL APPLICATION**

After it was found that colloidal sulphur prepared by the methods herein described was effective in inhibiting the germination of spores of certain economic fungi, as usually tested in the laboratory, it was next necessary to demonstrate the fungicidal value of these materials by practical field applications. Arrangements were made with Dr. L. F. Nickell of the Monsanto Chemical Works, East St. Louis, Ill., for the use of equipment to manufacture a sufficient quantity of materials for field tests.

The materials were prepared according to methods already described, with certain modifications to suit the available equipment. A 500-gallon wooden tub, fitted with a steam coil, agitators, cover, and a flue provided with a steam jet were used for the purpose.

The hypo-\(\text{H}_2\text{SO}_4\) method was necessarily modified, since the concentrated \(\text{H}_2\text{SO}_4\) could not be used in the tub because of the iron agitator and steam coil. The method used was as follows: Seven hundred pounds of hypo were dissolved in 75 gallons of water and the solution warmed with the steam coil to 50° C. Ten pounds of glue were dissolved by means of steam and this
was added to the hypo solution. One hundred pounds of commercial H$_2$SO$_4$, sp. gr. 1.84, were added slowly by means of a siphon. The mixture was then adjusted to P$_H$ 4.2 with sulfcide, 1 part to 5 parts of water. The agitator was kept in motion during the entire process.

The lime sulphur method was slightly modified as follows: Commercial lime sulphur solution was diluted with 4 parts of water and the mixture warmed to 40° C. Ten pounds of glue, previously dissolved by means of steam, were added for each 100 gallons of the diluted lime sulphur. The reaction of the lime sulphur mixture was then adjusted to P$_H$ 4.2 with HNO$_3$, 1 part to 20 parts of water. The agitator was kept in motion throughout the process. It was originally planned to allow the mixture to set over night with the agitator in motion in order to free the mixture of H$_2$S. Due to the short interval of time to which the writer was restricted in the use of the apparatus, another and more rapid method was used to eliminate the H$_2$S. Therefore, the mixture was heated to 70° C. and aerated several hours by means of an air pump. This aeration and heating unfortunately destroyed the stability of the solution to a considerable extent. Additional quantities of the mixture were prepared in a similar way by using 1 to 20 H$_2$SO$_4$.

The materials thus prepared were sent to the Missouri Agricultural Experiment Station, and to Adams and Chester Counties, Pennsylvania, to be used in tests being made for the control of apple diseases. Mr. R. C. Walton, of the Pennsylvania Agricultural Experiment Station, had charge of the field tests in Pennsylvania.

Due to the seasonal conditions very little disease appeared in the apple orchard at the Missouri Station. Therefore, no comparative results of value were obtained.

The results of the tests conducted in the commercial orchards of Pennsylvania were very favorable, particularly in the case of the preparation made from lime sulphur. These materials gave very efficient control of apple scab, a disease which was severe in Pennsylvania during that season. The details of these results will presumably be published separately (Walton, '26). The injury to foliage and fruit was a minimum in the plots sprayed
with these materials. The writer observed these plots in the late summer, and it was then evident that the foliage of the trees sprayed with the mixture prepared from lime sulphur and HNO₃ was of a much darker green color. Furthermore, the growers found the use of these materials satisfactory because of the ease of handling and applying.

Colloidal sulphur was made in the laboratory by the SO₂-H₂S method for field tests. The method used was the same as has already been described except that a 10-gallon glass carboy was used as a reaction tank for the SO₂ and H₂S. Large earthenware jars (especially made for the Chemical Department, Washington University) were used for H₂S generators. The product produced by this method was sent to the Michigan, Missouri, and New York Agricultural Experiment Stations, and to Adams County and Chester County, Pennsylvania, for field tests.

The results of these tests were not so promising as was anticipated, due probably to two factors: (1) The dilution of the sulphur used was probably too great; and (2), as has already been indicated, the sulphur crystallized in a short time after it was prepared. The technique of large-scale preparation, therefore, requires further detailed study.

**Conclusions**

1. Methods have been devised for preparing promising colloidal sulphur suspensions for use in practical spraying.
2. The stability of colloidal sulphur preparations was found to depend upon the method of mixing the chemicals, the temperature of solutions, and upon the introduction of protective colloids.
3. Of the emulsoid colloids that it has been practicable to employ, glue has proved to be the most effective in preserving the stability of colloidal sulphur suspensions.
4. The use of weak alkalies in adjusting suspensions prepared from hypo and H₂SO₄ was found necessary; and for this purpose Na₂CO₃ and Na₂HPO₄ gave the best results.
5. In preparing colloidal sulphur from lime sulphur, weak, rather than strong, solutions of HNO₃ and H₂SO₄ were more satisfactory.
6. The colloidal sulphur mixtures prepared by the methods
devised in the course of this work were found to be toxic to all forms of fungi tested under laboratory conditions.

7. Greenhouse tests with a variety of plants have shown that the products spread well and that they adhere to the foliage; at the same time they do not cause injury under the conditions tested.

8. Colloidal sulphur freshly prepared from SO$_2$ and H$_2$S was effective for the control of carnation rust in the greenhouse.

9. It would seem to be demonstrated that the toxicity of different forms of colloidal sulphur may be ascribed to different toxic substances. The properties of the toxic substances exhibited by colloidal sulphur prepared from SO$_2$ and H$_2$S are different from the toxic substance (pentathionic acid) exhibited by Young’s hydrophilic colloidal sulphur.

10. There is a gradual increase in toxicity of colloidal sulphur formed from SO$_2$ and H$_2$S, beginning at P$_H$ 4.2 and continuing to P$_H$ 5.4, while at higher P$_H$ values there is a rapid increase. Young’s hydrophilic colloidal sulphur exhibits the greatest toxicity in the range P$_H$ 4.2 to 5.4.

11. Colloidal sulphur prepared from SO$_2$ and H$_2$S in paste form will crystallize in about 1 month. This crystallization destroys the toxic property.

12. Colloidal sulphur preparations have greatest stability between P$_H$ 3.0 and 5.4. The loss of stability is accompanied by a destruction of the toxic property.

13. The toxic constituent of colloidal sulphur, when freshly prepared from SO$_2$ and H$_2$S, is slightly volatile and is liberated most rapidly at a higher P$_H$ than 4.2.

14. Desiccation and aeration do not destroy the stability or the toxic component of colloidal sulphur prepared from SO$_2$ and H$_2$S.

15. Colloidal sulphur prepared from SO$_2$ and H$_2$S is most toxic at higher temperatures.

16. When manufactured for field spraying tests the methods for preparing colloidal sulphur were modified to suit the equipment available. Prepared in this way, certain of the products gave excellent control of apple scab during the summer of 1924.
The writer takes great pleasure in extending thanks for many suggestions and criticisms to Dr. B. M. Duggar, who directed the work; to Dr. H. C. Young, of the Ohio Agricultural Experiment Station for many suggestions; to Dr. George T. Moore, for the privileges and facilities of the Missouri Botanical Garden; and to the Crop Protection Institute for the necessary funds and materials.

Graduate Laboratory, Missouri Botanical Garden.

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