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Studies on Lime-Sulphur-Mixture.

By

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I. Introduction

The history of lime-sulphur wash in the United States is so well known that it does not seem necessary to discuss about it here. The writer, however, thinks it advisable to give a brief account of the evolution of lime-sulphur, considering the character of the writer's present studies. According to QUAYLE[1], a sheep dip was tried on a fruit-tree in 1886 in California, and was found to be a good scalecide.

The mixture of that time was made from 80 pounds of lime, 100 pounds of sulphur, 20 pounds of sugar, 10 pounds of salt and 160 gallons of water. After being modified several times, the following was considered to be the standard formula in California in 1904:

lime	30 pounds
sulphur	15 pounds
salt	10 pounds
water	60 gallons

The lime-sulphur wash was used in several other states; and the formulas of preparation were not always the same. But, generally speaking, lime was used either as much as or sometimes more than sulphur, in preparing the

1) QUAYLE, H. J., Calif. Agric. Exper. Stat. Bull. 166, 1905.

mixture. Later, there appeared several kinds of commercial concentrated lime-sulphur on the market which could be stored for a long time and used after simply diluting with water. Effort was made in several agricultural experiment stations to make a similar concentrated lime-sulphur mixture and it was found that by using sulphur almost twice as much as lime a concentrated lime-sulphur mixture which was very similar to the commercial mixture, could be made. Thus, THATCHER[1] reported in 1906 that the best proportion of lime to sulphur was 1 : 1.95, if both materials were chemically pure.

Many investigations have been conducted since that time, and it has been known that both materials (lime and sulphur) are most economically used by adopting the above formula; that the percentage of calcium polysulphides in the mixture is the highest; and that the resulting mixture does not give rise to any sediment on standing.

It is needless to say that the study of the efficacy of the resulting mixture when it is actually used in an orchard against the scaleinsect, is highly important. Accordingly, there have been many experiments which were conducted to compare the commercial concentrated lime-sulphur with the standard homemade one, or to compare the homemade concentrated mixture with the standard homemade one. But, in reporting the results of experiments, it often happened that either the specific gravity of the original mixture or the strength of the diluted spray had not been given.

Moreover, in many cases the efficiency of the spray was not reported with figures, being stated simply "good," "very good," "excellent," etc.

But, on the whole, the effectiveness of the commercial lime-sulphur is believed to be equal to or somewhat higher than that of the standard homemade mixture which is made by using lime as much as, or sometimes more than sulphur. In as much as there has been only one experiment[2] in Japan, which was conducted with the object of comparing the various kinds of lime-sulphur, the writer attempted to study this point and at the same time, some of the more important properties of the lime-sulphur mixture. Though the results obtained thus far are by no means satisfactory, they will be stated in the following lines.

II. Comparison of Effectiveness between the Standard Lime-sulphur and the Concentrated Lime-sulphur.

Before entering into the writer's experiments, some preliminary statements are necessary. For the sake of convenience, the writer classifies the lime-

1) THATCHER, R. W., Washington Agric. Exper. Stat. Bull. 76, 1906.
2) ISHIKAWA, T., Special Rpt. Niigata-ken Agric. Exper. Stat. No. 2, (In Japanese).

sulphur mixtures roughly into the following two kinds:

The first kind.........Lime-sulphur mixtures in which lime and sulphur are used in equal amounts or lime is used in a larger quantity than sulphur.

The second kind.........Lime-sulphur mixtures which are made with about twice as much sulphur as lime, or sulphur used is more than twice the amounts of lime.

There are not much exact data as to how the lime-sulphur acts upon the scaleinsects. However, it is generally believed that calcium polysulphides in the mixtures are the principal active constituents. These compounds have a marked reducing power as well as power of depositing fine sulphur particles. These two properties of the polysulphides act injuriously upon the scaleinsects. Calcium thiosulphate has a slight killing effect, also. But, this compound is present in the mixture only in a small amount so that, the writer thinks, it does not seem to be an important constituent. Now, from the results of chemical reseach it has been known that the percentage of calcium polysuphides is the highest when the ratio of sulphur to lime is 2 : 1. For this reason the second kind of lime-sulphur has been considered by some investigators to be more effective in killing the scaleinsect than the first. However, the difference in percentage of the total sulphur as sulphides (including calcium polysulphides) between the first kind and the second kind is not very large. Indeed, THATCHER[1] went so far as to state that "the relative amounts of these two soluble compounds (calcium pentasulphide and calcium thiosulphate) which are present in the sprays prepared by any of the common formulas are practically the same, regardless of the proportion of ingredients used in boiling, etc."

The results shown in the following tables will show this relation.

Table I.

After Tartar.[2] Somewhat modified.*

Lime grms.	Sulphur grms.	Water c.cs.	Sulphide sulphur grms.	Polysulphide sulphur grms.	Thiosulphate sulphur grms.
110	110	450	3.60	12.50	4.96
60	110	450	3.60	12.40	3.84

1) THATCHER, R. W., Washington Agric. Exper. Stat. Bull. 76, 1906.

2) TARTAR, H. V., Oregon Agric. Exper. Stat. Resear. Bull. 3, 1914.

* Figures in the three tables are derived from the original data by calculation, supposing that the same quantity of sulphur had been used for a definite quantity of water in each kind of lime-sulphur in a table.

Table II.

After Van Slyke.[1] Modified.*

Lime lbs.	Sulphur lbs.	Water galls.	Sulphide & poly-sulphide sulphur lbs.	Thiosulphate sulphur lbs.
71.4	32.1	50	56.8	12.5
71.4	35.7	50	57.6	13.6
71.4	71.4	50	48.2	19.4

Table III.

After Thatcher.[2] Modified.*

Lime grms.	Sulphur grms.	Water c.cs.	Sulphide sulphur grms.	Thiosulphate sulphur grms.	Lime in solution grms.	*Lime in suspention grms.
15	15	500	11.94	2.80	7.60	7.50
15	9	500	12.20	2.60	7.67	1.30

As will be seen from the results shown above, the total amount of sulphur as sulphides (sulphide and polysulphides) does not show much difference in various mixtures made by different formulas, if we use the same quantity of sulphur for a definite amount of water when we prepare the mixtures. According to the writer's experiment the specific gravity of the first kind of lime-sulphur is only slightly higher than the second kind, if we prepare the two kinds under conditions stated above.

Therefore, if we use lime-sulphur as a spray diluted to the same specific gravity, a slightly larger amount of sulphur per unit volume of the diluted spray is contained in the second kind than in the first, strictly speaking. But, this difference would be very small when we use the mixture at an ordinary dilution.

For this reason the writer assumes that, if we compare the two kinds of lime-sulphur at the same specific gravity, we are treating the two spray solutions containing almost equal amounts of sulphur per unit volume of the diluted spray. As has been stated before, the reducing power of the polysulphides in the lime-sulphur is considered to be a very important property in killing the scaleinsect.

Now, SHAFER[3] showed that the reducing power of the spray solution within 18 hours after dilution is considerably larger when the lime-sulphur is diluted with whitewash than when it is diluted with pure water. These

1) VAN SLYKE, L. L., New York State Agric. Exper. Stat. (Geneva) Bull. 329, 1910.
2) THATCHER, R. W., Washington Agric. Exper. Stat. Bull. 76, 1906.
3) SHAFER, G. D., Michigan Agric. Exper. Stat. Tech. Bull. 11, 1911.

two points led the writer to study the difference in killing power between the two kinds of lime-sulphur mixtures. On searching for literature it has been found that many of investigators in the United States are of the opinion that the second kind is as effective as or slightly more effective than the first kind. As has been pointed out, exact data shown with fiigures are rather few. The following three studies will be briefly cited.

TAYLOR[1] published the results of his experiments in 1908. In his experiment 1 gallon of Rex lime-sulphur was diluted with 11 gallons of water and 10 pounds of lime was added to 50 gallons of the diluted spray. Mr. TAYLOR did not give the specific gravity of the original Rex lime-sulphur. Rex lime-sulphur has the specific gravity of 31 to 33 degrees Baumé. Supposing that the original mixture had been of 31° Baumé and calculating the amount of sulphur in one gallon of the diluted spray, we get approximately 10 pounds as the amount of sulphur which is present in 50 gallons of the diluted spray of Rex lime-sulphur. The homemade standard mixture was prepared with 15 lbs of lime, 15 lbs of sulphur and 50 gallons of water. Therefore, Rex lime-sulphur was used at a weaker concentration than the homemade one, as regards the amount of sulphur present in 50 gallons of the spray. The results of experiments showed that there was practically no difference in efficiency between the homemade mixture and Rex lime-sulphur mixture. In these experiments, however, lime was added to the diluted spray of Rex lime-sulphur, so that the results do not show strict comparison of the two kinds of mixtures.

FORBES[2] reported similar experiments. He compared the homemade standard mixture with several commercial lime-sulphur mixtures.

The homemade lime-sulphur was made according to the ordinary formula, i. e., 15 lbs of lime, 15 lbs of sulphur and 50 gallons of water. Rex lime-sulphur was used at the same dilution as in the TAYLOR's experiment. Professor FORBES did not show the specific gravity of Rex lime-sulphur, either. The results of his experiments showed that Rex lime-sulphur was slightly more effective than the homemade one. However, the difference was rather small, and Professor FORBES states in the summary that "the homemade solutions were equally effective to those ready-made ...."

MELANDER[3] conducted experiments on San Jose Scale with an object different from that of the writer's present study. But, in his experiments we have found a very good comparison of the effect of lime-sulphur. For the sake of convenience, the mixtures are named here the first, the second, the third and the fourth. The first and second were commercial concentrated lime-sulphur mixtures, and 3 gallons of the diluted solution of the first con-

1) TAYLOR, E. P., Missouri Fruit Exper. Stat. (Mountain Grove), Bull. 18, 1908.

2) FORBES, S. A., Illinois Agric. Exper. Stat., Bull. 180, 1915.

3) MELANDER, A. L., Jour. Econom. Entom., VIII, pp. 475—480, 1915.

tained 1 pound of sulphur; the second was diluted in such a way that 1 pound of sulphur was contained in 5 gallons of the spray. The third was a home-made concentrated mixture, and it was used at a rate of 1 pound of sulphur and 1/2 pound of lime in 5 gallons of the spray. The fourth was a homemade standard mixture and contained 1 pound of sulphur, and 2 pounds of lime in 5 gallons of the spray.

The examination of the results shows that the killing effect of the fourth (i. e., the standard homemade mixture) was unquestionably better than that of the other three in all other cases, excepting where an examination was made six weeks after the spraying in Clarkstone.

Finally, one experiment conducted in Japan will be cited. ISHIKAWA[1] conducted spraying experiment on San Jose Scale. Of various kinds of lime-sulphur mixtures used in his experiment, No. 1 was prepared with 8.3 lbs of lime, 4.15 lbs of sulphur and 5 gallons of water; No. 2 with 8.3 lbs of lime, 8.3 lbs of sulphur and 5 gallons of water; No. 3 with 4.15 lbs of lime, 8.3 lbs of sulphur and 5 gallons of water, and No. 4 with 4.15 lbs of lime, 9.96 lbs of sulphur and 5 gallons of water. All these were sprayed on San Jose Scale at a specific gravity of 3 degrees Baumé.

The average percentages of insects killed of two years' experiment were as follows:

No. 1 81%;	No. 2 90%;
No. 3 95%;	No. 4 97.5%.

In this experiment the homemade concentrated lime-sulphur seems to have been a little more efficient than the ordinary homemade standard one.

The writer's experiments.

In view of these conflicting results, the writer conducted similar experiments in 1917 and 1919. The writer prepared two kinds of lime-sulphur, i. e., the one in which equal amounts of lime and sulphur were used, and the other in which the amount of sulphur used was twice as much as the amount of lime in the former kind, lime and water used being the same in the two cases. The two mixtures were diluted to the same degree.

Experiment A. March 16th, 1917 Japanese pears infested with *Aspidiotus duplex* were sprayed with the two kinds of lime-sulphur diluted with water to 7.5 degrees Baumé. The trees were examined on April 6th. The results are shown in the following table.

1) ISHIKAWA, T., Special Report of Niigata-ken Agric. Exper. Stat., 2.

Table IV.

Spraying experiments with Japanese pear.

Tree No.	First kind of lime-sulphur			Second kind of lime-sulphur		
	Number of scales examined	Number of scales dead	% killed	Number of scales examined	Number of scales dead	% killed
No. 1	175	116	66.2	127	103	81.1
„ 2	146	113	77.3	196	138	70.4
„ 3	161	117	72.6	164	128	78.0
„ 4	127	121	95.2	207	147	71.0
„ 5	108	105	97.2	152	102	67.1
Average percentage killed			81.7			73.5

Experiment B. January 15th to 16th, 1919 persimmon-trees infested with *Aspidiotus duplex* were sprayed. The lime, as well as sulphur used for preparing the lime-sulphur was not of good quality. The mixture was prepared in the ordinary way practiced by orchardists on the farm. The formula were as follows:

The first kind.		The second kind.	
Lime	13 lbs	Lime	13 lbs
Sulphur	10 „	Sulphur	20 „

Water was used in such a quantity that the resulting mixtures showed the specific gravity of 7 degrees Baumé.

One or two of the trees sprayed were examined at times to know the best time of examination. A few of the scales sprayed began to drop from the trees about March 10th, and by May 15th a great many of scales had dropped. Still, there remained many scales on the trees and some of them were active and laying eggs.

Therefore, the last examination of all the trees sprayed was made on May 16th to 19th. The results are shown in the following table.

Table V.

Spraying experiments with persimmon-trees.

Tree No.	First kind of lime-sulphur			Second kind of lime-sulphur		
	Number of scales examined	Number of scales dead	Percentage killed	Number of scales examined	Number of scales dead	Percentage killed
No. 1	200	23	11 5	200	20	10.0
„ 2	200	13	6.5	200	19	9.5
„ 3	200	11	5.5	200	12	6.0
„ 4	200	36	18.0	200	15	7.5
„ 5	200	48	24.0	200	34	17.5
„ 6	134	12	8.9	200	18	9.0
„ 7	205	57	27.8	200	37	18.5
„ 8	177	27	15.2	200	30	15.0
„ 9	200	10	5.0	200	32	16.0
„ 10	200	12	6.0	—	—	—
„ 11	200	22	11.0	200	25	12.5
„ 12	200	20	10.0	200	8	4.0
„ 13	200	31	15.5	200	12	6.0
„ 14	115	9	7.8	200	16	8.0
„ 15	—	—	—	200	18	9.0
Average percentage killed			12.34			10.6

It will be seen from the results given in the table that the percentage of the scales killed by spraying varied within a rather wide range among different trees, and that the percentage of the scales killed were apparently very low.

As has been stated above, some of the scales killed by the spray had dropped, and these dropping scales do not seem to have occurred equally in different parts of a tree. This seems to account for such a variation in the percentage of the scales killed. Since this variation occurred almost equally on both kinds of trees, i. e., trees sprayed with the first kind of lime-sulphur and those sprayed with the second kind, the results could be used for comparing the effectiveness of the two kinds of lime-sulphur. The results in the two tables seem to show that the first kind of lime-sulphur was slightly more effective than the second. But, the difference was rather small, so that it would be safe to conclude that there was practically no difference between the two kinds of spray.

The writer sprayed peach-trees against *Diaspis pentagona* with the two kinds of spray. This scaleinsect was very resistant to the spray, and even in May only few of the species were dead. Many were evidently alive and laying eggs. The writer, therefore, did not examine the trees sprayed. It is apparent from this spraying that the lime-sulphur is not effective for all kinds of scaleinsects, as this has been already pointed out by several investigators.

III. Reducing Power of Lime-sulphur Spray.

It has been pointed out in a previous chapter that lime-sulphur has a strong reducing power, and that this property plays an important rôle in killing the scaleinsect. For this reason TARTAR[1] stated that the estimation of the reducing power would be a good method of evaluation of lime-sulphur.

Therefore, the writer undertook to study the reducing power of lime-sulphur and to compare the two kinds of lime-sulphur in regard to this property.

The method of study was as follows:

Preparation of lime-sulphur. Lime and sulphur which were almost chemically pure, were used. In the first kind of lime-sulphur 15 grams of lime and 500 c.c. of water were used, and in the second kind the amount of lime was reduced to 7.5 grams, all other conditions being the same. Sulphur was mixed with a small portion of water of 70°—80°C, and lime was slaked with the hot water. These two were poured into a flask, and the hot water was poured into the flask until the volume of the mixture was about 500 c.c. The mixture was heated in a bath of boiling water; it was stirred at times by shaking the flask, and the flask was kept in the bath for one hour. The flask was then taken out, cooled until the mixture inside was 15°C. The water lost by evaporation was added, and the volume of the mixture was made accurately to 500 c.c.

It was then filtered, and the specific gravity of the filtrate was measured. This clear lime-sulphur was poured into a bottle about to its mouth, stoppered airtight and kept for study. The specific gravity of this original clear mixture was 5.4° to 5.6° Baumé in the first kind, and 5.2° to 5.4° Baumé in the second kind. A poriton of this lime-sulphur mixture was diluted to 100 times its volume accurately with distilled water which had been previously boiled and cooled. A certain quantity of this diluted mixture was used for determination of reducing power. The process of making the mixture, as well as measuring the reducing power, was quite the same in the two kinds of lime-sulphur. Therefore, a unit volume of the diluted solution must have

1) TARTAR, H. V., Oregon Agric. Exper. Stat., Resear. Bull., No. 3, 1914.

contained practically the same amount of sulphur in each kind. In almost all cases the solution was tested for its reducing power on the day of its preparation. The reducing power of the diluted solution was tested with $\frac{N}{10}KMnO_4$ solution. The determination of the reducing power was made in two ways:

First experiment. The diluted lime-sulphur solution with potassium permanganate solution added, was heated for a certain time; then, the reducing power was determined.

Second experiment. The diluted lime-sulphur was stirred for a while after the addition of the permanganate, and the reducing power was determined immediately. The results of these experiments are shown in tables VI and VII. The figures in the tables show the number of c.c. of the potassium permanganate reduced.

The first experiment: In this case 5 c.c. of the diluted lime-sulphur was used.

Table VI.

Reduction with heating.

Samples	The first kind of lime-sulphur	The second kind of lime-sulphur	Samples	The first kind of lime-sulphur	The second kind of lime-sulphur
No. 1	3.97	4.76	No. 5	5.96	5.69
„ 2	4.46	5.91	„ 6	4.51	4.46
„ 3	5.87	5.49	„ 7	5.09	5.04
„ 4	6.03	6.06	„ 8	4.99	5.05
Average				5.11	5.60

Supposing that the determination had been made under the normal condition (with regard to pressure and temperature), and calculating from the results shown in table VI, we get approximately 58 c.c. of oxygen which is absorbed by 1 c.c. of the original lime-sulphur mixture. It will be seen that the reducing power of lime-sulphur is quite strong.

The second experiment: In this case 10 c.c. of the diluted solution was used for the determination of the reducing power.

Table VII.

Reduction without heating.

Samples	The first kind of lime-sulphur	The second kind of lime-sulphur	Samples	The first kind of lime-sulphur	The second kind of lime-sulphur
No. 2	3.67	3.35	No. 7	4.73	4.74
„ 3	4.30	4.07	„ 8	4.82	4.87
„ 4	3.85	3.46	„ 9	5.09	5.10
„ 5	3.84	3.52	„ 10	5.11	5.11
„ 6	3.76	3.38	„ 11	5.12	5.03
Average				4.02	3.87

It will be seen that the reducing power of lime-sulphur as determined by the second method is almost half as great as that determined by the first method. Therefore, we can infer that in the second experiment the oxidation of lime-sulphur did not proceed so far as in the first experiment.

According to the results shown in table VI, the second kind of lime-sulphur seems to have a slightly stronger reducing power than the first, while, with the second experiment the reverse was the case. The difference was rather small in each experiment, so that we may perhaps conclude that there is practically no diffence between the two kinds of lime-sulphur with regard to their reducing power, if we prepare the two mixtures by using the same amount of sulphur for a definite quantity of water.

IV. Dissolving Power of Lime-sulphur towards Scales.

Scales of the coccids have been considered to be a kind of wax for a long time. MAULIK,[1] however, showed that the scale of *Lepidosaphes ulmi* does not dissolve in the ordinary solvents of wax, and that it contains nitrogen. Therefore, he considers that the scale of this coccid is not wax.

SHAFER[2] found that lime-sulphur had power to soften the peripheral part of a scale which was newly secreted, and that the solution made the scale stick on the tree. But, he found that the body of a scaleinsect which had become comatose by the action of lime-sulphur was rarely seen to have been actually touched by lime-sulphur.

The writer observed that lime-sulphur can wet the scale when it is sprayed on it. But the lime-sulphur does not seem to pass through the scale. The material which constitutes a scale has a higher specific gravity than lime-sulphur at an ordinary dilution. Yet, scales do not sink into the solution, even if we purposely immerse them in it. Now, we put scales into a small vial which contains lime-sulphur solution in its lower half, close its mouth with a rubber-stopper with a short glass tube inserted through it. If we suck out the air in the vial through this tube, and stir at times the lime-sulphur with scales, we see that scales now sink into the solution after we have stopped the pumping out of air. It will be seen, therefore, that the scale is of higher specific gravity than the lime-sulphur solution, that the scale, however, is of loose texture and contains air in it.

This air prevents the solution from going through them, when the lime-sulphur is sprayed on scales. With the method just described the writer tested whether lime-sulphur has the power to dissolve scales. The scales of *As-*

1) MAULIK, S., Bull. Entom. Resear., VII (1916—1917), pp. 267—269.
2) SHAFER, G. D., Michigan Agric. Exper. Stat., Techn. Bull., No. 11, 1911.

pidiotus duplex were collected; about one half gram of them was dried and weighed; and they were immersed in lime-sulphur for a period of from 1 to 14 days. After that they were taken out, thoroughly washed, dried and weighed again. With various lime-sulphur solutions of specific gravity 7—12 degrees Baumé, the writer repeated these experiments eight times.

But, the weight of scales after immersion into the solution did not decrease in any case.

Scales were dipped in xylene, benzene and turpentine oil, and it was found that none of the above solvents dissolved the scales. From these results it seems, as Mr. MAULIK says, that the scale of this coccid is not an ordinary wax, and that lime-sulphur does not possess dissolving power towards the scale.

V. Some Remarks about the Examination of Scales sprayed with Lime-sulphur.

The examination of the scales sprayed with lime-sulphur wash looks at first sight to be an easy matter. In reality, however, it is not. In the first place the writer raises this question:

By what criterion can we judge exactly whether a scaleinsect is dead or not? This is an important question, and at the same time a very difficult problem. PIPER[1] stated with regard to San Jose Scale that "two phenomena were relied upon to determine the death of the insect: 1st. The change in color of the body from pale yellow to a dull orange........., but a considerable percentage of the insects' bodies do not show this change in color. 2nd. The shrivelling of the body and apparent drying, etc."

MELANDER[2] wrote about the insect, "Not only is it extremely difficult, if not impossible, to decide just when a scaleinsect dies, but the last count was further vitiated by certain scales dropping from the branches, etc. During the reaction the protoplasm of the insect changes in appearance, becoming viscous and in color luteous yellow until further change darkens the protoplasm to a brown and changes its consistency to an oily meal. Just when this transition point occurs where this reaction ceases to be reversible, and death ensues is unknown, but in interpreting the results the insect at the viscous, luteous stage is considered dead."

In short, the easiest way to tell whether a scaleinsect is living or dead, seems to be to judge the change in color of the insect's body. However, two difficulties accompany this. 1. Even though it is true that change in

1) PIPER, C. V., Washington Agric. Exper. Stat., Bull. 56, 1903.

2) MELANDER, A. L., Loc. cit.

color occurs after the insect dies, there are many gradations of shades in color from bright yellow to dark yellow (or to brown), so that at a certain shade we do not know whether the insect should be classed as the living or as the dead. 2. It takes a certain time for the change in color to appear.

Now, if we wait till the change in color of all the dead insects appears, dropping of scales will have begun before that time.

In *Diaspis pentagona*, *Aspidiotus duplex* and San Jose Scale, the writer observed that not only dead scales, but also some of the scales of apparently living insects dropped from the tree. If the scale of a living insect drops, the insect body will also drop after a while. This dropping of scales begins to occur at different intervals after spraying in different species; thus, in San Jose Scale, scales begin to drop about two weeks after spraying.

Besides these two, there is another important factor. Even in one species, the time when the effect of spraying appears is sometimes different in different localities. The exact reason of this is not known. It may be due either to the difference in climate or to different races (or varieties) of the same species, or to both of these. Thus, ISHIKAWA[1] sprayed against San Jose Scale in November, and he found that over 81% had died 10 days after spraying.

The writer sprayed Japanese pear-trees with lime-sulphur of 5 degrees Baumé against San Jose Scale on December 4th, 1918 and obtained the following results.

Table VIII.

Spraying against San Jose Scale.

Date of Exam.		No. 1	No. 2	No. 3	No. 4	No. 5
Dec. 14	Number of scales examined	394	—	—	—	—
	Number of scales killed	47	—	—	—	—
	Percentage killed	11.9	—	—	—	—
Dec. 24	Number of scales examined	413	150	357	76	470
	Number of scales killed	97	11	31	10	29
	Percentage killed	23.4	7.3	8.7	—	6.1
Jan. 3, 1919	Number of scales examined	432	—	429	—	313
	Number of scales killed	34	—	38	—	19
	Percentage killed	7.8	—	8.8	—	6.7
Jan. 23, 1919	Number of scales examined	448	—	—	—	383
	Number of scales killed	50	—	—	—	40
	Percentage killed	11.1	—	—	—	10.4

1) ISHIKAWA, T., Loc. cit.

As will be seen in table VIII, the writer found that 23.4% was the highest percentage of the insects killed on December 24th, and that after this time the percentage killed apparently did not increase. It was considered that this apparent stop in increase was due to dropping of dead scales. At any rate, the appearance of the results of lime-sulphur spraying in the writer's experiment was much slower than in the experiment of Mr. ISHIKAWA.

A similar fact, i. e., the apparent difference in resistance to lime-sulphur spray of San Jose Scale in different localities, was observed by Mr. MELANDER.[1)]

In determining the appropriate time of examination of the trees sprayed, we must take these facts into consideration. The writer thinks that in case of San Jose Scale the examination should be made at least about 20 days after spraying under the conditions prevailing in Kurashiki. In examining the scaleinsects sprayed, the writer considered those insects dead, the color of which was dark orange or brown.

VI. Enzymes in the Body of living and dead Scaleinsects.

The writer stated in a previous chapter that some of the scaleinsects which had been sprayed, which, however, looked apparently alive, showing no change of color, dropped from the trees. The writer sprayed against *Diaspis pentagona* infesting the peach or the cherry. In this case a great many of them showed no change in color even in May and some of them were undoubtedly alive; for they laid many eggs, when their attachment to the tree began to loosen. Are all the scaleinsects which do not show change in color, really alive? Or, does the color not change on account of certain circumstances, even after the insect is dead? Such questions are of course very important, if we want the results of examination of the trees sprayed, to be accurate.

Now, SHAFER[2)] reported that the activity of catalase and oxidase in the body of an insect killed with gasoline or hydrocyanic acid gas, had greatly decreased.

CROCKER and HARRINGTON[3)] studied the enzymes contained in grass seeds and found that, in the Johnson grass, there is a close relation between catalase and respiration of the seeds; that the older the seeds the weaker is the activity of catalase, and that there is no close relation between the vitality

1) MELANDER, A. L., Loc. cit.
2) SHAFER, G. D., Michigan Agric. Exper. Stat., Techn. Bull., No. 21, 1915.
3) CROCKER, W. and HARRINGTON, G. T., Jour. Agric. Resear., XV, pp. 137—174, 1918.

of the seeds and the catalase in them.

In view of these facts the writer attempted to study the activities of oxidase and catalase in the body of scaleinsects which had been sprayed with lime-sulphur.

A. Oxidase.

For testing the activity of the oxidase guaiac tincture was found best suited for the writer's purpose. The species studied was *Diaspis pentagona.*

Experiment I. A certain number of scaleinsects were crushed in a small agate mortar, and to this insect tissue pulp (or juice) was added a small quantity of guaiac tincture.

(a) Living insects. 5 living insects were crushed, a little guaiac tincture was added to this juice, and this mixture was kept in a thermostat of 25°C for 10 minutes. A beautiful blue color appeared. Next, 1 insect was crushed, and treated in the same manner. A blue color developed.

(b) Insects which had been sprayed with lime-sulphur about one month before the experiment. These insects were treated in the same manner and were found to have almost the same oxidizing power.

(c) Insects killed by fumigation. Scaleinsects which were fumigated about a week before the test, and which turned brown, were treated in a like manner, and were found to possess oxidizing power, also.

Comparison of the oxidizing power of the living scaleinsects and that of the insects killed by fumigation was next made. But, it was found that there was practically no difference.

Experiment II. In this experiment the oxidizing power of water in which insects were immersed for a certain length of time was studied. It is a known fact that the protoplasm of a living organism has semipermeability to a certain substance, and that, when the organism is dead, the protoplasm becomes sometimes permeable to the same substance. In view of this fact, Dr. OSUGI suggested to the writer to study whether a change in permeability to oxidase occurs, when the scaleinsect dies. If the protoplasm and the epithelium of an organ, (such as the alimentary canal), should lose their semipermeability to oxidase, oxidase would diffuse out into the water in which the insects were immersed.

The method of study, therefore, consists in immersing insects in water and testing the water for oxidizing power. The method of study was also suggested by Mr. OSUGI.

The insect studied was *Diaspis pentagona*.

(a) A certain quantity of water was taken in a small test tube, and 20 living insects were immersed in it. 7 of such test tubes, each containing 20 insects were prepared. After two hours, the clear water covering the scaleinsects was decanted into another small test tube. To these 7 test tubes containing the water was added a small quantity of guaiac tincture, and 3 hours later there appeared a pale blue color in each test tube.

At the same time, the same process was carried on with the scaleinsects which had been killed by fumigation. 7 test tubes containing water in which these insects were immersed, showed blue color, also. All these test tubes (14 in all) were kept in a thermostat till next day, and the depth of the blue color was compared between the living and dead insects. A similar experiment was repeated again. The results of these experiments are shown in table IX.

Table IX.

Comparison of oxidizing power between water in which living insects were immersed (A) and that in which fumigated insects were immersed (B).*

A			B		
Deeper blue than B	Equal to B	Less deep blue than B	Deeper blue than A	Equal to A	Less deep blue than A
0	1	6	6	1	0
0	0	8	8	0	0

(b) Scaleinsects sprayed with lime-sulphur were compared with living insects in a similar way. One hour after the addition of guaiac tincture, no color developed in any of the tubes containing the water in which living insects were immersed. The experiment was conducted twice. The results of comparison two hours after the addition of guaiac tincture are shown in the following table.

Table X.

Comparison of oxidizing power between water in which living insects were immersed (A) and that in which insects sprayed with lime-sulphur were immersed (B).

A			B		
Deeper blue than B	Equal to B	Less deep blue than B	Deeper blue than A	Equal to A	Less deep blue than A
1	8	3	3	8	1
1	15	1	1	15	1

From the results shown in tables IX and X, it will be seen that a larger amount of oxidase diffused out into water, when the insects killed by fumigation were immersed in water than when living insects were immersed; and that there was practically no difference in the quantity of oxidase dissolved

* Figures in this and the next table show the number of test tubes.

in water, between the case where living insects were immersed, and the case where the insects which had been sprayed with lime-sulphur were immersed. It would seem from these results that the scaleinsects which had been sprayed with lime-sulphur previously, which, however, did not show change in color at the time of the experiments, were alive.

B. Catalase.

The activity of catalase was studied quantitatively. A certain number of insects were crushed in an agate mortar; a certain quantity of water was added. This insect tissue pulp was filtered, and a certain quantity of hydrogen peroxide was added to this filtrate.

The mixture was kept in a thermostat for a certain period of time. Then, the quantity of hydrogen peroxide which remained undecomposed was titrated with potassium permanganate; the amount decomposed by catalase could be thus determined. Commercial hydrogen peroxide was carefully neutralized, and was used at a strength of 1%.

All insects were of course treated in the same way. The results of experiments are shown in the following table. The figures show the number of c.c. of hydrogen peroxide decomposed.

Table XI.

Catalase in the body of the scaleinsect.

(I) Diaspis pentagona.

(a) Living insects.

	H_2O_2 added	H_2O_2 decomposed	Remarks
	2	1.93	50 insects were used at a time.
	2	1.62	30 insects.
	3	2.37	30 „

(b) Insects sprayed with lime-sulphur.

Number of days after spraying	H_2O_2 added	H_2O_3 decomposed	Remarks
30*	2	1.78	Color of insects not changed. 30 insects not used at a time.
30*	2	1.92	Ditto.
10	2	1.77	„
22	2	1.78	„
30	2	1.77	„

* These two figures are somewhat doubtful.

(*Table XI continued.*)

(c) Insects killed by fumigation.

Number of days after fumigat.	H_2O_2 added	H_2O_2 decomposed	Remarks
3	2	1.76	30 insects used. No change in color.
5	2	1.70	Turned brown.
7	2	1.65	Ditto.
32	2	0.94	,,
62	2	0.47	,,

(II) Aspidiotus duplex.

(a) Living insects.

	H_2O_2 added	H_2O_2 decomposed	Remarks
	2	all	30 insects used. H_2O_2 added was not sufficient.
	4	3.7	
	4	3.8	

(b) Insects sprayed with lime-sulphur.

Number of days after spraying	H_2O_2 added	H_2O_2 decomposed	Remarks
11	2	1.87	No change in color.
23	4	3.20	12 out of 65 turned brown. Insects which turned brown were mixed with living ones in this proportion.
32	4	2.32	Dead and living were mixed, and used.
50	3	all	Insects which showed no change in color were used. H_2O_2 was insufficient.

(c) Insects killed by fumigation.

Number of days after fumigat.	H_2O_2 added	H_2O_2 decomposed	Remarks
1	2	all	H_2O_2 added not sufficient.
3	2	all	Ditto.
11	2	1.70	Some turned brown; these were used, mixed with those which showed no change in color.
15	4	2.80	Mostly turned brown.
31	4	2.73	All turned brown.
72	4	1.47	Ditto.

(*Table XI continued.*)

(d) Insects killed by hot water immersion.

(1) 10 minutes at 50°C.

Number of days after immers.	H_2O_2 added	H_2O_2 decomposed	Remarks
1	2	1.69	No change in color.
31	4	2.07	All turned brown.

(2) 5 minutes at 60°C.

Number of days after immers.	H_2O_2 added	H_2O_2 decomposed	Remarks
1	2	1.93	No change in color.
5	2	1.76	Ditto.
19	4	2.66	All turned brown.

(e) Insects killed by starvation and freezing.

	H_2O_2 added	H_2O_2 decomposed	Remarks
	4	2.22	All changed in color.

All these figures in this table are the average of 5 or 6 determinations.

The results in the table can be summarized as follows:

1. *Diaspis pentagona* which had been sprayed with lime-sulphur, which, however, did not show any change in color of body, had almost the same reducing power as the living insects.
2. In *Aspidiotus duplex*, some insects were killed by lime-sulphur and showed change in color after a certain length of time. When, however, we used for study only those individuals which did not show change in color, the activity of the catalase was almost as great as that of living insects.
3. In case of insects killed by fumigation, the activity of catalase was a little weaker when it was tested 7 or 10 days after fumigation than when it was studied of living insects. With increase in number of days after fumigation, the activity of the catalase decreased gradually. However, the rate of this decrease was rather small, so that we could not find a considerable decrease unless after one month or more had elapsed.
4. In cases where insects were killed by heat which was considered not to affect the catalase, and also in cases where insects were killed by freezing and starvation, the activity of the catalase decreased after a certain period of time.

From these results it is apparent that the insects which had been sprayed with lime-sulphur, whicn, however, did not change in color, seemed to be alive, and that we shall not be able to tell more quickly by the activity of catalase or of oxidase than by change in color, whether scaleinsects are alive or not. However, it may not be impossible to tell whether scaleinsects are dead or not, before change in color appears, by utilizing the change in permeability of the protoplasm and epithelium of a scaleinsect to catalase or oxidase.

VII. Summary.

1. The comparison of the effectiveness of the two kinds of lime-sulphur mixture was made. The first kind was made using equal quantities of lime and sulphur. In preparing the second kind the same amounts of sulphur and water as in the first kind were used, the amount of lime, however, being reduced to one half the amount of lime in the first kind. There seems to be practically no difference in effectiveness between these two kinds of lime-sulphur mixture.
2. With regard to the reducing power towards potassium permanganate the two kinds of lime-sulphur showed practically no difference.
3. Lime-sulphur mixture seems to have no dissolving power towards the scales of *Aspidiotus duplex*.
4. When scaleinsects had been sprayed with lime-sulphur, attachments of the scales were loosened a certain time after the spraying, and some of the scales dropped from the tree. Not only the scales of the dead insects, but also those of the apparently alive insects dropped.
5. After the scaleinsect was dead, the activities of oxidase and of catalase in the tissue of the insect decreased gradually. The results of study made using this phenomenon seem to show that the insects which did not change in color a certain time after the spraying, were not dead at that time.

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