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Virus Diseases of Plants

Purification of the Virus of Mosaic Disease of Tobacco

C. G. VINSON

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Virus Diseases of Plants

Purification of the Virus of Mosaic Disease of Tobacco

C. G. Vinson

Vinson[1] recorded a method of precipitating the virus from juice of diseased plants by adding an aqueous solution of safranin. Later[2] a simple and rapid method of decomposing this precipitate was described. The method of decomposing the safranin-virus precipitate consists of suspending it in water, then adding Lloyd's alkaloidal reagent, which removes the safranin. When sufficient Lloyd's reagent has been added the safranin is removed, leaving the virus in the aqueous phase.

Effect of Increasing Concentration of Safranin on the Precepitation of the Virus

When the first safranin precipitate was obtained there was always the question whether the virus was precipitated and if so to what extent was it precipitated. The supernatant liquid from the safranin precipitate failed to infect plants except in rare instances. It was early realized that this failure might be due to the presence of the safranin, since dilute solutions of safranin are very injurious to the plant, even when dropped on unwounded areas. The safranin, therefore, might kill the tissues so rapidly and over sufficient area as to prevent access of the virus to living cells.

To obtain further information as to the completeness with which safranin precipitates the virus, increasing amounts of one per cent aqueous safranin solution (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, and 20 cc.) were added to 25 cc. of juice from diseased plants (See Table I). After centrifuging, the supernatant liquid in each case was decanted and the safranin removed from the decanted supernatant liquid with Lloyd's reagent. In the removal of the safranin, it was noted that the same amount of Lloyd's (one gram) was effective in removing the safranin from each sample.

An aliquot from each decolorized supernatant liquid was diluted with an equal volume of water and 100 plants inoculated with the preparation, using five pins and puncturing one leaf per plant. To another like preparation were added 100 milligrams of activated charcoal and another 100 plants inoculated. In this experiment 2500 plants were

1 Vinson, C. G. Precipitation of the Virus of Tobacco Mosaic. *Science* 66, 357-358 (1927).

2 Vinson, C. G. Mosaic Disease of Tobacco. V. Decomposition of the Safranin-Virus Precipitate. *Phytopathology* 22, pp. 965-975, Dec. 1932.

TABLE 1.—THE EFFECT OF SAFRANIN CONCENTRATION ON PRECIPITATION OF THE VIRUS FROM JUICE OF DISEASED PLANTS

No. cc. of a 1% aqueous safranin solution added to 25 cc. of juice from diseased plants	Preparation obtained by decolorizing supernatant liquid from safranin precipitate with Lloyd's reagent				Checks	
	5 cc. of preparation plus 5 cc. redistilled water		5 cc. of preparation plus 5 cc. redistilled water plus 0.1 gram of activated charcoal			
	No. plants inoculated	No. plants diseased	No. plants inoculated	No. plants diseased	No. plants inoculated	No. plants diseased
1	100	78	100	92	100	0
2	100	76	100	94	100	0
3	100	64	100	86	100	0
4	100	27	100	69	100	0
5	100	2	100	22	100	0
6	100	0	100	38	100	0
7	100	0	100	1	100	0
8	100	1	100	1	100	0
9	100	0	100	2	100	0
10	100	0	100	0	100	0
15	100	0	100	0	100	0
20	100	0	100	0	100	0

used, including 100 check plants. The juice samples all came from the same lot of plants, placed in the refrigerator at the same time, thawed and expressed at the same time. The results indicate that with increasing safranin concentration the precipitation of the virus progresses toward completion, and that when a certain safranin concentration is reached the precipitation is approximately, if not quite, complete (perhaps much more nearly complete than is the removal of sulfate ion with barium ion or vice versa). Our preparations with two to four grams of total solids in solution per 10,000 grams of water have been shown to be fully as infectious as the original juice. Our qualitative test, then, for the virus is far more sensitive than our test for barium or sulfate ion. The results in Table 1 also further illustrate the effect of adding finely divided charcoal to a virus preparation.

The amount of precipitate obtained by adding 1 cc. of a one per cent aqueous safranin solution was one-half cc., but the amount of precipitate progressively increased with increasing concentration of safranin until 8 cc. had been added. The packing was approximately the same in each of the first eight tubes since they were centrifuged together. If the virus is adsorbed on another precipitate this may indicate that adsorption of the virus is complete just at the point where the precipitation of the other substance by safranin is complete. The experiment was run the latter part of August and the diseased plant material had been under outdoor conditions for about four weeks. Juice from field grown diseased plants requires a still greater amount of safranin to complete the precipitation of the virus.

Acetone Precipitation of the Virus from the Fraction Obtained on Decomposing the Safranin-Virus Precipitate

Vinson[1] and Vinson and Petre[3] have reported the precipitation of the virus from juice of diseased plants by adding two volumes of acetone to one volume of the juice. When this precipitation is carried out at a temperature sufficiently low, the virus is not inactivated; on the other hand, when the precipitate is dissolved in a volume of water equal to that of the original juice it has been demonstrated that the solution of the acetone precipitate apparently is fully as infectious as the original juice. In other words such precipitation of the virus from juice of diseased plants is rather complete.

The above acetone procedure was applied to the virus fraction obtained on decomposing the safranin-virus precipitate. Two volumes of cold acetone were added to one volume of the virus fraction in the cold and centrifuged at once. The precipitate packed sufficiently to allow complete decantation of the supernatant liquid. The precipitate was dispersed in a volume of water equal to that of the original juice. It dispersed readily in each experiment to give an opalescent solution that was found to be less infectious than the original virus fraction from which obtained (Table 2). The supernatant liquid from the acetone precipitate was decanted and diluted at once to a concentration of acetone which would not inactivate the virus and plants inoculated with the diluted supernatant liquid. In each case the diluted supernatant liquid demonstrated considerable infective power, again showing that the precipitation of the virus was by no means complete.

Solid and ash determinations on the precipitate and supernatant liquid indicate that the major part of the ash remained in the supernatant liquid, but even so the ash made up about thirty per cent of the precipitate.

TABLE 2.—PRECIPITATION OF THE VIRUS WITH ACETONE FROM THE VIRUS FRACTION OBTAINED ON DECOMPOSING THE SAFRANIN-VIRUS PRECIPITATE WITH LLOYD'S REAGENT

Virus fraction obtained from decomposing the safranin-virus precipitate with Lloyd's reagent, undiluted		Solution of the acetone precipitate resulting from the addition of 2 volumes of acetone to 1 volume of the virus fraction obtained on decomposing the safranin-virus precipitate, undiluted		Supernatant liquid from the acetone precipitate			Checks	
No. plants inoculated	No. plants diseased	No. plants inoculated	No. plants diseased	Dilution	No. plants inoculated	No. plants diseased	No. plants used	No. plants diseased
		100	69	1 to 2	100	31	100	1
		100	59	1 to 2	100	26	100	1
100	52	100	41	1 to 3	100	13	100	1

1 Vinson, C. G. Precipitation of the Virus of Tobacco Mosaic. *Science* 66, 357-358 (1927).
3 Vinson, C. G. and Petre, A. W. Mosaic Disease of Tobacco. Contributions Boyce Thompson Institute for Plant Research. Vol. 1, pp. 479-503 (1929).

Reprecipitation of the Virus with Safranin

Since the virus fraction obtained by decomposing the safranin precipitate contains only a small fraction (2 to 8 per cent) of the solids of the original juice, reprecipitation of the virus from this fraction with safranin offered the possibility of further reducing the amount of irrelevant or accompanying substances. The carrying down of accompanying substances by occlusion or adsorption should not be so great as from the original juice.

The two preliminary experiments designed to test the possibility of reprecipitating the virus with safranin indicated that the supernatant liquid from the second precipitate contained very little virus, and the virus fraction obtained on decomposing the second precipitate was highly infectious. More elaborate experiments were then planned, designed to show relatively how much of the virus could be accounted for in the virus fraction obtained on decomposing the second precipitate. A total of about twelve thousand plants (11,900) was used in these experiments.

Out of a total of eleven comprehensive experiments (Table 3), three gave results in which the virus fraction obtained by decomposing the second safranin precipitate was not as infectious as the corresponding respective fraction from the first safranin precipitate; in three of these experiments the results were inconclusive; and in five of the eleven experiments the virus fraction from the second precipitate apparently was fully as infectious as that from the first precipitate. The supernatant liquids from the second precipitate after being decolorized with Lloyd's reagent, all showed very low, or no, infective power.

In one of the eleven experiments the decomposition of the second safranin precipitate was carried out in 20 per cent acetone as well as in purely aqueous solution.

The last four experiments were so planned as to give information on the influence of freezing on the infective power of purified virus preparations. In these experiments the virus fraction was prepared, an aliquot taken, diluted with an equal volume of water and one hundred plants inoculated before and after adding activated charcoal. At the same time another aliquot of the purified virus solution (usually 25 cc.) was placed in a refrigerator at about 5 degrees Fahrenheit for 24 hours. The frozen aliquot was thawed on the centrifuge at 3000 r.p.m.. This latter procedure reduced the number of larger suspended particles. An aliquot was then taken from the centrifuged supernatant liquid, diluted with an equal volume of water and one hundred plants

Table 3.—Results Obtained on Reprecipitation of the Virus with Safranin

The juice expressed from diseased plants, diluted with an equal volume of water				Virus solution prepared by removing the safranin with Lloyd's reagent from a suspension of the first safranin-virus precipitate				Virus solution prepared by removing the safranin with Lloyd's reagent from a suspension of the second safranin-virus precipitate				Preparation obtained by decolorizing with Lloyd's reagent the supernatant liquid from the second safranin-virus precipitate				Checks	
Without activated charcoal		With activated charcoal added		Without activated charcoal		With activated charcoal added		Without activated charcoal		With activated charcoal added		Without activated charcoal		With activated charcoal added			
No. Inocu-lated	No. Dis-eased	No. Inocu-lated	No. Dis-eased	No. Inocu-lated	No. Dis-eased	No. Inocu-lated	No. Dis-eased	No. Inocu-lated	No. Dis-eased	No. Inocu-lated	No. Dis-eased	No. Inocu-lated	No. Dis-eased	No. Inocu-lated	No. Dis-eased	No. Used	No. Dis-eased
								50	24	50	43	50	1	50	1		
								50	14	50	39	50	1	50	0		
100	10	100	23	100	31	100	53	100	43	100	24	100	0	100	0	300	0
100	10	100	31	100	45	100	36	100	21	100	21	100	1	100	1	100	0
							In 20% Acetone										
100	19	1 00	25	100	29	100	42	100	23	100	47	50	2	50	3	100	0
							Aqueous Solution										
								100	33	100	41						
100	29	100	79	100	48	100	67	100	40	100	45	50	1	50	2	100	0
100	25	100	47	100	27	100	25	100	24	100	41	50	0	50	0	400	0
100	27	100	59	100	25	100	51	100	31	100	37	50	0	50	0	300	0
100	12	100	40	100	39	100	75	100	31	100	53	50	0	50	0	100	0
				100	56	100	59	100	63	100	74						
100	31	100	55	Frozen:				Frozen:				50	6	50	5	100	0
				100	43	100	70	100	55	100	69						
				100	71	100	76	100	69	100	79						
100	4	100	15	Frozen:				Frozen:				50	4	50	3	100	0
				100	27	100	72	100	43	100	87						
				100	51	100	3	100	55	100	67						
100	11	100	35	Frozen:				Frozen:				50	1	50	4	100	1
				100	36	100	53	100	65	100	71						
				100	13	100	22	100	14	100	29						
100	5	100	21	Frozen:				Frozen:				50	0	50	1	100	0
				100	10	100	25	100	19	100	22						

inoculated before and after adding activated charcoal. The results show that freezing reduced the infective power of the fraction from the first precipitate uniformly, *but the infective power was restored when suspended material was again added in the form of activated charcoal.* Freezing and centrifuging apparently may or may not reduce the infective power of the virus fraction from the second precipitate, but in any case the infective power after the addition of activated charcoal was increased until equal to that of the fraction from the first precipitate.

Attention is also called to the fact that the addition of activated charcoal to the *juice* usually results in a greater percentage increase in infective power than results when activated charcoal is added to purified virus fractions. The latter may show as great an increase in infective power, but not as often as in the case of the juice. Infrequently the addition of charcoal to the juice may cause a decrease in infective power. This happens more often in the case of the purified virus fractions and is typified by the results in the fifth and tenth experiments of Table 3.

A few solid and ash determinations have been made on the virus fractions from the first and second precipitates. The results of these determinations indicated a reduction in total solids of 20 to 40 per cent, and sometimes more, in case of the virus fraction from the second safranin precipitate as compared with the corresponding fraction from the first precipitate. The percentage of solids of the original juice appearing in the virus fraction from the safranin precipitate depends upon the total solid content of the juice. Juice in the summer, especially from field plants, is higher in solid content and, as one would expect, a higher percentage of solids is carried down by the safranin precipitate.

Precipitation of the Virus by a Solution of Aluminum Sulfate and also by Acidification

A few drops of a normal solution of aluminum sulfate solution suffice to precipitate the virus fraction almost completely from the preparation obtained on decomposing the safranin precipitate with Lloyd's reagent. The aluminum precipitate packs sufficiently on centrifuging to allow complete decantation of the supernatant liquid. The supernatant liquid may be colored, but when precipitation is complete it is water clear, and so far very little infective power has been demonstrated in it (Table 4 and 5). The precipitate is small in amount, usually brown or amber in color; although practically colorless preparations may be obtained. An even better preparation of the virus fraction has been obtained by adding small amounts of $N/_1$ acetic acid

TABLE 4.—PRECIPITATION OF THE VIRUS FRACTIONS BY ADDING A SOLUTION OF ALUMINUM SULFATE TO A VIRUS PREPARATION OBTAINED ON DECOMPOSING THE SAFRANIN PRECIPITATE

Experiment No.	Original juice from diseased plants		Virus fraction by decomposing the safranin precipitate		The virus fraction precipitated from the purified virus preparation by adding a few drops of N / 1 $Al_2(SO_4)_3$, 18 H_2O solution				Check Plants	
					The aluminum sulfate ppt. suspended in dilute neutral phosphate solution		The supernatant liquid from the aluminum sulfate ppt.			
	Without Charcoal	With Charcoal	Without Charcoal	With Charcoal	Without Charcoal	With Charcoal	Without Charcoal	With Charcoal	Number Used	Number Diseased
1	16	34	9	33	22	33	1	0	200	0
2	20	39	15	48	17	28	0	0	200	0
3	15	38	18	31	20	44	0	1	100	0
4	20	47	17	39	46	50	0	0	100	0
5	41	76*	52	73	37	77	2	5	200	1
6	63	83*	34	67	40	64	6	9	100	0
7	30	83*	30	67	25	63	1	5	100	1
8	43	92*	50	67	37	66	1	5	200	0
9	26	81*	29	67		62	0	0	200	1

*Lloyds and charcoal added to the juice.

TABLE 5.—COMPARATIVE INFECTIVE POWER OF THE ORIGINAL JUICE, THE VIRUS FRACTION OBTAINED ON DECOMPOSING THE SAFRANIN PPT. AND THE $Al_2(SO_4)_3$ PPT. FROM THE DECOMPOSED SAFRANIN PRECIPITATE

	5 cc. of original juice from diseased plants plus 5 cc. of redistilled water				5 cc. of virus prepn. (from decomp. safranin ppt.) plus 5 cc. redistd. H_2O		The aluminum ppt. suspended in dilute neutral PO_4 5 cc. plus 5 cc. redistd. H_2O		Supernatant liquid from aluminum ppt. 5 cc. plus 5 cc. redistd. H_2O		Check Plants	
Experiment No.	No Addition	With Lloyd's	With Activated Charcoal	With Lloyd's and Activated Charcoal	Without Charcoal	With Charcoal	Without Charcoal	With Charcoal	Without Charcoal	With Charcoal	Number Used	Number Diseased
1	41	46	56	76	52	73	37	77	2	5	200	1
2	63	79	81	83	34	67	40	64	6	9	100	0
3	30	72	67	83	30	67	25	63	1	5	100	1
4	43			92	50	67	37	66	1	5	200	0
5	26			81	29	67		62	0	0	200	1
6				89		82					100	1
7				89		55					200	0
8				96		89					200	0
9				77		61-97*					100	0
10				97		82					200	0
11				67		72-74*					200	0
12				99		95					850	6
13			88	92		80					850	6
14				60		53					200	0
15				76		69					200	3
16				77		51					100	0

9200 plants inoculated

*After a second gram of Lloyd's reagent was added to clear up the turbid preparation.

to the virus preparation obtained on decomposing the safranin precipitate. The precipitate so obtained also packs well on centrifuging and usually is less highly pigmented than the corresponding aluminum precipitate. Like the aluminum precipitate it is highly infectious when suspended in dilute neutral phosphate solution.

This means of taking the virus out in a solid fraction from purified preparations is much more satisfactory than by precipitation with alcohol or acetone in that it is believed to be more highly selective and may be carried out at room temperature; whereas precipitation with alcohol or acetone must be carried out in the cold if activity is to be retained. Precipitation with acid or with aluminum sulfate solution is, indeed, a very satisfactory means of concentrating the virus fraction.

These precipitates may be readily dispersed in aqueous media with hydrogen ion concentration sufficiently low (especially in phosphate or acetate solution of pH 7.0 or a little above). The infective power of such a dispersion is high (Table 4 and 5).

The virus may also be precipitated by carefully adding acid to the neutral phosphate eluate (from the lead precipitate of the virus). Acidification is especially effective in removing the virus from the concentrated (in vacuo) neutral phosphate eluate.

The precipitate may be cream colored or colorless, is very low in ash, and gives a curdy precipitate on being heated. By lowering the hydrogen ion concentration the precipitate is readily dispersed. The nitrogen content is about 15 or 16 per cent, and it gives the regular protein tests.

The preparation obtained by decomposing the safranin precipitate with Lloyd's reagent has generally proven more infectious than the original juice from which the preparations have been obtained. The juice and the preparation were, however, not comparable so far as amount and character of suspended materials were concerned. Table 5 does show that when Lloyd's reagent is added to a sample of juice, in the same proportion as that used in obtaining the purified preparation through the safranin procedure, then the infective power generally becomes greater than that of the preparation. The data in this table also indicate that the aluminum sulfate precipitate of the virus (from the purified preparation obtained through the safranin procedure) compares favorably in infective power with the preparation obtained on decomposing the first safranin precipitate.

The nitrogen content of the final fractions, from juice of both diseased and healthy plants, obtained through the lead acetate precipita-

TABLE 6.—NITROGEN ANALYSIS

Sample	Juice 25 cc. samples		Mgs. of nitrogen in the neutral PO_4 eluate of the lead precipitate from 50 cc. of juice		
	From diseased plants	From healthy plants	From diseased plants	From healthy plants	Blank
1	19.0	18.9	1.5	0.2	0.2
	18.5	18.4	1.5	0.2	0.3
2			1.4	0.3	0.2
			1.4	0.2	0.25
3	20.0	21.6	1.5	0.2	0.2
	19.55	21.1	1.5	0.2	0.3
4			1.4	0.2	0.2
			1.3	0.2	0.15
5	17.8	9.4	1.4	0.15	0.3
	17.5	9.3	1.3	0.1	0.3
6			1.0	0.3	0.2
			1.0	0.2	0.2
7	17.4	10.3	1.2	0.2	0.3
	17.0	10.3	1.2	0.2	0.3
8			1.25	0.2	0.2
			1.3	0.2	0.2
9	17.2	10.6	1.3	0.2	0.1
	17.4	11.4	1.4	0.2	0.1
10			1.5	0.2	0.2
			1.5	0.2	0.2
11	18.0	9.1	1.5	0.2	0.1
	18.2	9.5	1.4	0.2	0.1
12			1.4	0.2	0.2
			1.3	0.2	0.2
13	16.2	11.0	1.4	0.2	0.2
	15.8	10.9	1.4	0.2	0.1
14			1.3	0.2	0.2
			1.3	0.2	0.2
15	17.4	9.7	1.3	0.3	0.2
	17.2	9.8	1.35	0.25	0.2
16			1.3	0.2	0.1
			1.4	0.3	0.15
17	16.9	12.4	1.3	0.3	0.2
	16.9	12.1	1.3	0.3	0.2
18			1.3	0.2	0.15
			1.3	0.3	0.1
19	15.25	10.65	1.2	0.2	0.2
	15.60	10.3	1.2	0.3	0.2
20			.7	0.2	0.2
			.7	0.2	0.2

tion method of Vinson and Petre[3] is given in Tables 6 and 7. A solution of lead acetate when added to juice from healthy plants produces a precipitate which contains nitrogen as well as does that from juice of diseased plants. After elutriation of such precipitates with an $M/_3$ solution of monopotassium ortho phosphate and subsequent suspension in dilute ($M/_{15}$) neutral phosphate solution, the supernatant liquid, obtained on centrifuging of the fraction from healthy plants contains very little or no nitrogen. With larger samples, however, the presence of small amounts of nitrogen no doubt could be demonstrated. The fraction from 500 cc. of juice from diseased plants was found to contain about 10 milligrams of nitrogen.

The virus precipitate produced by adding a solution of lead acetate to a virus fraction was never successfully decomposed with hydrogen sulfide when the virus precipitate was suspended in plain water. Lead sulfide formed but the virus was always inactivated.

Table 8 gives the data obtained from experiments where such a lead precipitate of the virus was suspended in a dilute solution ($M/_{15}$)

3 Vinson, C. G. and Petre, A. W. Mosaic Disease of Tobacco. Contributions Boyce Thompson Institute for Plant Research. Vol. 1, pp. 479-503 (1929).

TABLE 7.—NITROGEN ANALYSIS

Sample	Juice		Mgs. nitrogen in the lead precipitate from 25 cc. of juice after extraction with acid PO_4 followed by extraction with neutral PO_4		
	From diseased plants	From healthy plants	From diseased plants	From healthy plants	Blank
1	16.7	18.9	0.9	0.6	0.1
	16.3	18.4	0.8	0.55	0.2
2			0.5	0.7	0.2
			0.5	0.7	0.25
3	17.9	21.6	0.3	0.5	0.25
	18.6	21.1	0.5	0.5	0.2
4			0.5	0.5	0.2
			0.2	0.5	0.15
5	14.2	9.4	0.5	0.45	0.2
	13.2	9.3	0.4	0.5	0.25
6			0.3	0.5	0.2
			0.45	0.5	0.2
7	13.0	10.3	0.5	0.5	0.2
	13.1	10.3	0.5	0.5	0.2
8			1.0	0.6	0.2
			1.0	0.6	0.2
9	14.2	10.6	0.7	0.4	0.15
	14.75	11.4	0.75	0.5	0.2
10				0.4	0.2
				0.4	0.2
11	13.6	9.1	0.4	0.4	0.1
	13.55	9.5	0.4	0.4	0.2
12			0.45	0.4	0.2
			0.5	0.3	0.2
13	16.1	11.0	0.5	0.5	0.2
	16.4	10.9	0.45	0.45	0.1
14			0.4	0.4	0.2
			0.5	0.4	0.1
15	11.9	9.7	0.5	0.4	0.2
	11.9	9.8	0.5	0.4	0.2
16			0.5	0.4	0.2
			0.4	0.4	0.2
17	14.8	12.4	0.4	0.5	0.2
	14.3	12.1	0.4	0.5	0.2
18			0.5	0.3	0.2
			0.5	0.4	0.2
19	11.4	10.65	0.6	0.5	0.2
	11.5	10.3	0.5	0.5	0.2
20			0.55	0.5	0.2
			0.6	0.5	0.2

of neutral phosphate then hydrogen sulfide passed into the suspension for ten minutes. A black precipitate of lead sulfide always formed. After passing in the hydrogen sulfide the tubes were aspirated ten minutes to drive out most of the excess hydrogen sulfide. The lead sulfide precipitate often settled out nicely on centrifuging, leaving clear supernatant liquids. In some cases, as shown in Table 8, where the lead sulfide did not settle satisfactorily on centrifuging, Lloyd's reagent was added. After mixing and centrifuging again the supernatant liquid was usually apparently free of lead sulfide.

Inoculations with a suspension of the lead sulfide precipitate and with the supernatant liquid indicated that most of the infective power was present in the supernatant liquid.

In order to note effect of time on the condition of the virus in the various preparations, some tests were made at intervals on the infectivity of such fractions (Table 9). It is interesting to note that the

TABLE 8.—LIBERATION OF THE VIRUS FROM THE LEAD PRECIPITATE BY MEANS OF HYDROGEN SULFIDE

Experiment Number	Virus fraction obtained on decomposing the safranin precipitate		.3 cc. of a neutral $Pb(OAc)_2$ solution (200 gms. in 1000 cc. H_2O) added to 25 cc. of the virus preparation								Supernatant liquid from lead precipitate 5 cc. to 5 cc. redistilled HIO		Check Plants	
			Suspension of the lead precipitate											
			Neutral phosphate suspension centr.				Neutral phosphate suspension +HIS centr.							
			5 cc. of supernatant liquid plus 5 cc. of redistilled HIO		5 cc. of a suspension of precipitate plus 5 cc. redistilled HIO		5 cc. of supernatant liquid plus 5 cc. of redistilled HIO		5 cc. of a suspension of precipitate plus 5 cc. redistilled HIO					
	Without Charcoal	With Charcoal	Without Charcoal	With Charcoal	Without Charcoal	With Charcoal	Without Charcoal	With Charcoal	Without Charcoal	With Charcoal	Without Charcoal	With Charcoal	Number Used	Number Diseased
1	23	35	6	25	1	2					1	2	100	0
			*				19	40	7	18	12	26		
2	19	27					25	25	11	9	1	0	100	0
*3	30	26					28	40	2	12	18	21	100	0
4	37	43	*				18	30	7	13	5	0	200	0
			+				24	34	0	6	1	7		
+5	36	47					35	41			1	2	200	0
+6	23	26					16	45			1	0	400	0
							±18	26	5	2				
+7	33	45					29	43	--	--	0	1	300	0
							±30	48	2	10				
+8	9	33					29	29	--	--	2	0	200	0
							±20	31	6	4				

*Precipitated from acid PO_4 of about pH 4.5
+From acetate buffer of pH about 4.5
±1/10 th gm. of Lloyd's used to carry down the pbS
100 plants inoculated in each case.

TABLE 9.—EFFECT OF AGING UPON THE INFECTIVE POWER OF VIRUS PREPARATIONS.

Preparation	Date Prepared	Plants Inoculated 5/20/33		Plants Inoculated 5/24/33		Plants Inoculated 6/10/33		Plants Inoculated 7/20/33		Plants Inoculated 8/29/33		Plants Inoculated 11/25/33	
		No. Inoculated	No. Diseased	No. Inoculated	No. Diseased	No. Inoculated	No. Diseased	No. Inoculated	No. Diseased	No. Inoculated	No. Diseased	No. Inoculated	No. Diseased
Lead acetate precipitate from the second safranin precipitate Susp. in PO_4	5/20/33	10	10					10	9	10	9	10	9
Lead acetate ppt. from 2nd safranin in PO_4 sat. with H_2S maintained for entire period	5/21/33	10	10					10	6	10	10	10	10
$Al_2(SO_4)_3$ ppt. from 2nd safranin Susp. in PO_4	5/20/33	10	10	10	10	10	9	10	1	10	4		
First safranin precipitate	5/20/33											10	10

lead and safranin precipitates after standing six months still yielded highly infectious preparations. Also, a lead precipitate suspended in neutral phosphate and the system maintained in a saturated condition with hydrogen sulfide for six months apparently held up in infective power. It is possible, however, that the infective power of the aluminum sulfate precipitate was reduced on standing.

SUMMARY

With increasing concentrations of safranin the virus of tobacco mosaic from juice is progressively carried down until precipitation is approximately complete.

Acetone precipitation of the virus in the fraction obtained on decomposing the safranin precipitate has not proven satisfactory.

Reprecipitation of the virus with safranin may be successfully carried out.

A few drops of a normal solution of aluminum sulfate suffice to precipitate the virus from the fraction obtained on decomposing the safranin precipitate. Careful acidification will also precipitate the virus from such a fraction. Such precipitates may be readily dispersed by lowering the hydrogen ion concentration.

About ten milligrams is the content of nitrogen in the virus fraction obtained from 500 cc. of juice of diseased tobacco plants through the lead acetate solution procedure. Very little or no nitrogen was detectable in corresponding samples from juice of healthy plants.

Hydrogen sulfide may apparently be employed to decompose the lead-virus precipitate when the latter is suspended in a dilute solution of neutral phosphate.

The lead and safranin precipitates after standing six months still yielded highly infectious fractions.