THESIS

THE CHEMOTHERAPY OF CARNATION MOSAIC VIRUS

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY RALPH BAKER

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BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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Ralph Baker has demonstrated that he has an acceptable reading knowledge of German scientific literature within his selected field.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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Chapter I

INTRODUCTION

There is a recent trend to improve the quality of commercial carnations by selecting disease-free plants for the establishment of mother blocks. Though some progress has been made in attaining this goal, difficulty has been experienced in the selection of plants within certain popular varieties such as Miller's Yellow, in which the incidence of mosaic infection is almost 100 percent. The maintaining of the selected stocks in a virus-free condition is also difficult because the carnation mosaic virus is so easily transmitted by contact. It was apparent, therefore, that study was needed to test the possibilities of inactivating the virus in infected plants. This would aid in maintaining stock free from mosaic and would prevent its spread to healthy plants.

The problem

Can the carnation-mosaic virus be chemically inactivated *in vivo*?

Problem analysis.--Before answering the major question, it is desirable to answer the following:

1. What chemicals will inactivate the carnation-mosaic virus *in vitro*?

2. Which chemicals are suitable for the inactivation of carnation-mosaic virus *in vivo*?

Deliminations.[1]-- Tests will be applied to determine what chemicals will inactivate the carnation-mosaic virus *in vitro* and *in vivo*. Phytotoxic tests will be applied to carnations (*Dianthus caryophyllus* L.) and sweet william (*Dianthus barbatus* L.). All tests will be limited to the laboratory and greenhouses at Colorado A & M College.

[1]During the course of this investigation parallel studies were made in cooperation with other workers (1) to determine the effect of carnation mosaic on the yield and quality of carnation, (2) insect and root transmission of mosaic and streak, (3) indexing of disease-free mother blocks of commercial carnation varieties, (4) fungicidal soil treatment for controlling Fusarium wilt of carnations, (5) the carrier phenomenon of *Fusarium* in carnations, (6) and factors determining the development of bacterial wilt of carnations. Out of these investigations the following publications resulted in which the writer participated:

Baker, R. R. and W. D. Thomas, Jr. Chemical inactivation of the carnation-mosaic virus *in vivo*. Jour. Colo.-Wyo. Acad. Sci. 4(1): 51. 1949.

_____ and W. D. Thomas Jr. Root transmission of the carnation-mosaic virus. Jour. Colo.-Wyo. Acad. Sci. (in press).

Chapter II

REVIEW OF LITERATURE

Carnation mosaic has been observed for some time on many commercial varieties of carnations (Dianthus caryophyllus L.). As early as 1914 Peltier (15) described a disease of carnations the symptoms of which seem similar to those of present day carnation mosaic. Lamkey (13), among others (9, 16, 18), believed the disease to be of a virus nature. It was not until 1943, however, that the carnation mosaic virus was demonstrated to be of virus origin (8). Recent investigation has shown that carnations may be infected with at least two distinct virus diseases: mosaic and streak. (12). A disease known as yellows is caused by a combination of the viruses causing mosaic and streak. The characteristic symptom of mosaic is a slight mottling of the leaves, while plants affected with streak have yellowish or reddish spots and streaks paralleling the leaf veins. Both mottling and streaking may occur when a plant has yellows. Mosaic is spread rapidly by mechanical contact while streak evidently is transmitted only by insects or by grafting.

The recognition of symptoms is a major problem in the study of carnation viruses. Due to the narrow leaf of the carnation, symptoms often are not readily seen, and in many cases are overlooked because of masking. Symptoms may become severe in the spring, however, when aggravated by unfavorable nutrient conditions and heavy growth. The mottling is usually more pronounced in the young leaves

than in the older ones. (12).

As the sweet william (D. barbatus L.) has been found to be a host of both mosaic and streak, its use as a test plant has been suggested (7). Because of its broader leaf and relative freedom from confusing physiological disturbances, the symptoms of both viruses can be distinguished more easily. A later development has indicated that the viruses can be detected by means of fluorescense in ultra violet light. (26).

The use of chemicals for the inactivation of viruses in vitro has been a generant field from the beginnings of virus research. The first extensive experiments were performed by Allard (1, 2). Of the many chemicals tested he found that acids, bases, potassium permanganate, zinc chloride, copper sulphate, antiformin and strong solutions of alcohol or formaldehyde destroyed the infectivity of the tobacco mosaic virus. Stanley (20) found that trypsin produced a decrease in the infectivity of tobacco-mosaic virus over a wide pH range. The infectivity of the virus was regained by heat, dilution, or by digestion for the removal of trypsin. Later Stanley discussed the influence of different hydrogen-ion concentrations on the infectivity of the virus (21). Stanley (22) also has reported results on the effect of 110 different chemicals on tobacco-mosaic virus. Some increased, others decreased infectivity to varying degrees while still others had no effect. Johnson (11), using special techniques, studied the effect of 41 chemical substances harmful to the infectivity of the

tobacco-mosaic virus. He found that reactivation was possible in 28 cases by treatments with salts or acids in the proper concentration. The suggestion was made that the term "inactivation" be used in cases in which the virus can be reactivated, and that "destruction" be used when the virus cannot be reactivated.

Stoddard (23) recently has opened up an entirely new field of study for the control of some virus diseases. He found that the X-disease virus of peach was inactivated merely by soaking diseased peach buds in water solutions of quinhydrone, urea, calcium chloride, zinc sulfate, and various sulfa compounds. Preliminary treatments of soil with these chemicals have indicated some success.

Takahashi (25), using the detached leaf tissue technique and assying the virus concentration in Nicotiana glutinosa, showed that malachite green would inhibit the increase of tobacco-mosaic virus in living cells. That the host is involved in the inactivation of the virus is indicated by the fact that malachite green has no effect on the virus in vitro.

In a series of preliminary studies, Rumley (17) treated mosaic-infected carnation cuttings with nine chemicals including five sulfa compounds. Of these calcium chloride, hydroquinone, and zinc sulphate were found to inactivate the virus. However, sulfa compounds were found to be very toxic to an unrooted carnation cutting.

Best (4, 5, 6,) found that the invasion of tobacco

leaves by the tomato spotted-wilt virus causes an accumulation of an organic fluorescent substance called scopoletin (6-methoxy-7-hydroxy 1:2 benzo-pyrone). This compound is present in small quantities as a normal constituent of healthy tobacco plants, but it is produced in large quantities as the result of virus activity (4). As the concentration of the fluorescent substance is highest where the concentration of virus is greatest, the substance may well act as an inactivant (6).

Chapter III

MATERIALS AND METHODS

The carnation mosaic virus becomes inactive in a short time in expressed plant juice and decoctions of infected plant tissue. (12). Due to this apparent instability, its inactivation by chemicals would seem highly probable. The possible inactivation qualities of calcium chloride, zinc sulfate, and the various sulfa compounds were suggested by the preliminary work of Rumley. (17). Notable among the more widely used and effective chemicals showing therapeutic activity are many metallic compounds (24). As Dithane Z78 and sodium selenate are generally known to contain metallic elements and to be non-toxic to plants in the proper concentrations, they were included in these experiments. The use of malachite green as an inactivator was postulated by the recent studies of Takahashi (25). Scopoletin (6-methoxy-7-hydroxy 1:2 benzo-pyrone) was included in these tests for the reasons set forth in Chapter II.

In all experiments two hosts of the carnation-mosaic virus were used. White Patrician was used as a susceptable carnation variety (Dianthus caryophyllus) while sweet william (D. barbatus) was used as a test plant. In some instances it was found that symptoms were difficult to read on the former due to masking and physiological disorders. As a result, symptoms in carnations were never relied upon as the sole criterion for virus infection.

Carnations used in the experiments were rooted cuttings taken from a mother block of eleven plants. Over a period of one year repeated tests showed that extracts from these plants could infect at least one test plant in four by means of abrasion inoculation.

Inoculation techniques.--All D. barbatus plants were grown from seed in a greenhouse fumigated against insects at least once a month and in which night temperatures were approximately 60° F. It has been found[1] that inoculation with carnation-mosaic virus is more successful under moist atmospheric conditions than under dry. Ivanoski (10). has also found that conditions of high humidity favor the expression of virus symptoms. Therefore, inoculations were performed when the atmospheric moisture concentration within the greenhouse was high and after the first true leaves were fully developed. Four or five days preceding inoculation, the test plants were watered with a solution of ammonium sulphate (1 oz./2 gals.) in order to produce better symptoms and a higher percentage of infection as suggested by the work of Spencer (19). Between inoculations, the hands were washed with strong soap, dried, rinsed in 95% alcohol, and dried again with a different towel. Carborundum (400 mesh) was then sprinkled on the leaves. A young lateral shoot of the carnation to be tested for the presence of virus was macerated between the thumb and forefinger and the juice rubbed on the leaves of the test plant. As 100 percent transmission is seldom accomplished, the development of symptoms on any of the four inoculated plants

[1] W. D. Thomas, Jr. Unpublished data.

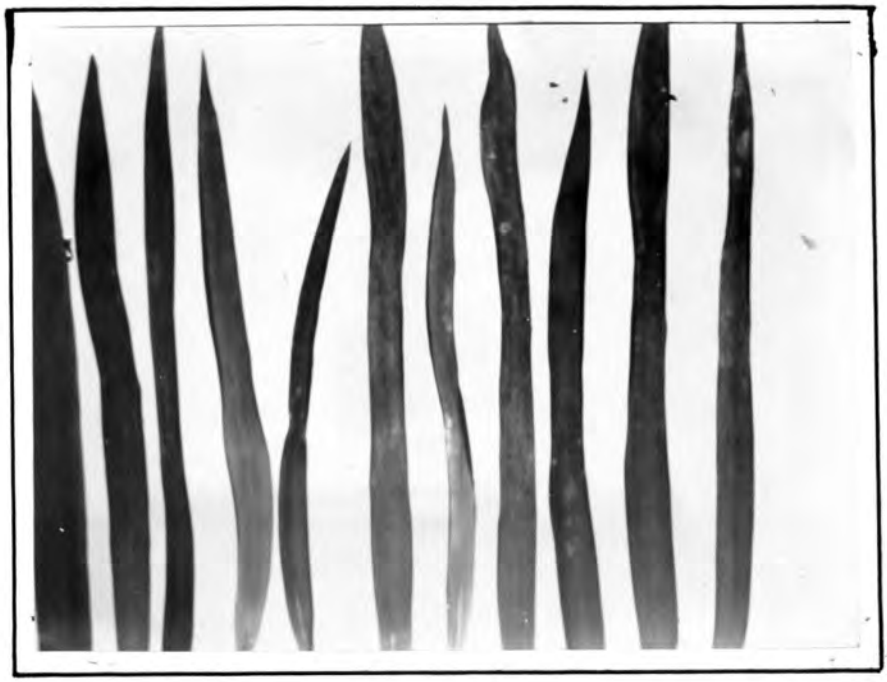

Fig. 1.--Illustration of symptoms of the carnation-mosaic virus on the leaves of the mother block of 11 D. caryophyllus plants. All carnation cuttings used in these experiments were obtained from these plants.

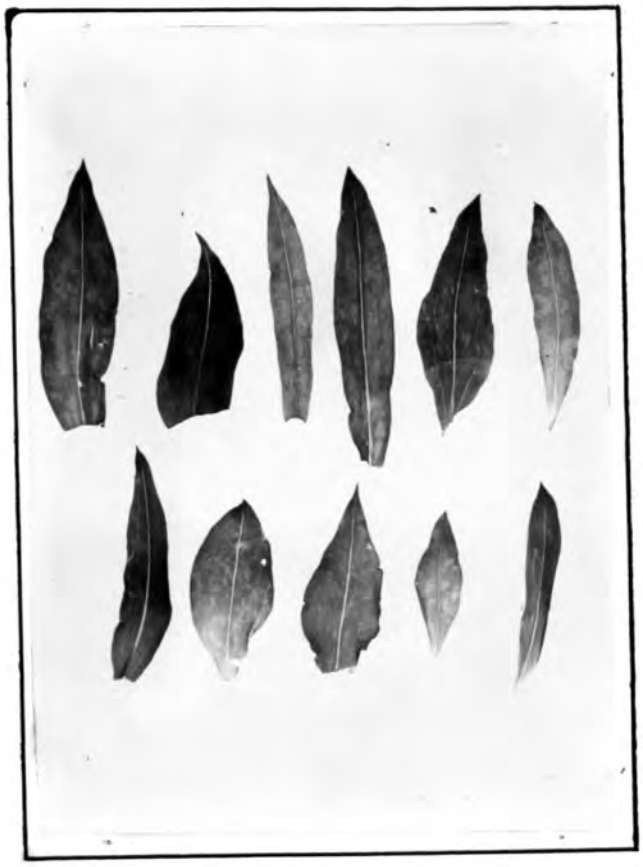

Fig. 2.--Varying degrees of infection of the carnation-mosaic virus on D. barbatus. These leaves were inoculated with an extract from the D. caryophyllus plants illustrated in Fig. 1.

was considered as evidence of virus activity in the original carnation.

To test the efficacy of sterilization procedures between respective inoculations, infectious plant juice was liberally applied to the hands, which were rinsed with strong soapy water and alcohol in accordance with the procedures outlined previously. A small amount of distilled water was applied to the thumb and forefinger. Carborundum was sprinkled on the leaves of nine test plants which were then rubbed lightly with the thumb and forefinger. After 30 days no evidence of virus activity in the nine plants was noticeable, while 17 plants out of 24 plants inoculated with the infectious juice became infected.

In vitro tests.--In an exploratory experiment, injection of chemicals was attempted by means of a thread-wick drawn through the stem of the plant to be treated (3). The lower end of the wick was suspended in a water solution of the chemical to be tested. The solution was thus taken up by the plant by means of the wick. As this technique was found to be unsatisfactory and impractical, it was abandoned.

A study of the inactivation qualities in vitro of the following chemicals was undertaken: proliferol (diureide of glyoxyllic acid), sulfamerazine, malachite green, potassium phosphate, potassium chlorate, calcium chloride, sodium diethyl dithiocarbamate, sulfasuxidine, trypan blue, sulfaguanidine, sulfathalidine, sodium selenate, zinc sulphate, and Dithane Z78 (zinc

ethylene bisdithiocarbamate). Cuttings from infected carnation plants were placed in a refrigerator and frozen overnight. The plant material was then macerated through a meat grinder and the juice expressed through two layers of cheesecloth. The juice was centrifuged at 3500 r. p. m. for one hour. With vigorous stirring, the test chemicals were added to 2 ml., or larger portions, of the centrifuged juice at a concentration of 1 per cent by weight. In addition, proliferol was tested at a 10 percent concentration. These mixtures were tested immediately for virus activity by passage into D. barbatus plants.

In vivo tests.--Two methods were used in attempting to inactivate the virus in vivo. In the first, D. barbatus were grown in steamed soil in flats measuring 13 in. x 20 in. Each flat contained 96 plants spaced 1 in. x 1 in. divided into four sets. The following chemicals in solution were applied: calcium chloride, zinc sulphate, sodium selenate, proliferol, malachite green, trypan blue, Dithane Z78, p-aminobenesulfonylamide, sulfaguanidine, and sulfamerizine. These were applied at the concentrations given in table 3 at the rate of one liter of solution daily per flat for eight days. Two sets of plants in each flat were inoculated five days before and after the beginning of soil treatments, respectively, with juice extracted and centrifuged from frozen infected plants. Another set was inoculated ten days after treatments were begun. The fourth set was left as an untreated check.

In the second method, diseased carnation plants were

grown in sand and were watered with a nutrient solution, as described by Mussenbrock (14), containing the chemical listed previously at the concentrations given in table 5. Glazed crocks, seven inches in diameter, were equipped with a drainage tube and were filled with sand. Two infected carnation plants were grown in each jar. Each test inactivant contained within the nutrient solution was applied to five pots. Only the nutrient solution was applied to five other pots as checks. The solutions were poured through the sand in these jars and the excess solution was collected by means of the drainage tubes as illustrated in Fig. 3. Solutions were reused each day for 14 days without renewal. At the end of the test, D. barbatus plants were inoculated to determine virus activity.

A modification of the latter method involved the application of nutrient solution containing the test chemicals to carnation plants growing in a flat measuring 20 in. x 24 in. Each flat contained 16 mosaic-infected carnation plants with a spacing of 5 in. x 6 in. Here, again, sand was used as a growing medium. In this case no provision was made for collecting the excess liquid. Nutrient solutions were applied every other day, except during cloudy weather, at three-fourths the strength used in the preceeding test over a period of 30 days. During cloudy weather it was found that some modification of watering schedules was desirable due to the decreased intake of solutions by the plants. For the first 20 days the plants were topped when at least three

Fig. 3.-- Method of application of nutrient solution containing inactivating chemicals in the second inactivation *in vivo* experiment.

nodes had developed. Test plants were inoculated as before.

Tests involving scopoletin.--A special experiment was set up to determine the inactivating properties of scopoletin *in vitro*. The scopoletin used was in the form of purified crystals obtained from Dr. L. Marion, National Research Council of Canada. Three sets of *D. barbatus* growing in 3-inch pots and containing 81 plants in each set were placed in the greenhouse. As scopoletin is photosensitive, two sets were subjected to continuous light while a third was completely shielded by a cardboard carton which eliminated light prior to inoculation. After 72 hours each set was inoculated by the abrasive technique with the following solutions: (1) scopoletin in 15 ml. of infectious centrifuged plant juice, (1:4000), (2) a saturated solution of scopoletin in 15 ml. of distilled water, (1:4000) and (3) 15 ml. of infectious centrifuged plant juice. The light proof cover was removed from the set kept in the dark prior to inoculation and placed for 72 hours over one of the sets exposed to light prior to inoculation. Treatments are further shown in table 7.

Chapter IV

EXPERIMENTAL RESULTS

In vitro tests.--The results of chemical inactivation tests *in vitro* are recorded in tables 1 and 2. In the first test (table 1) 24 *D. barbatus* plants were inoculated with each chemical dissolved in the infectious plant juice and were observed for virus symptoms after 28 days. In this experiment 1 percent proliferol, malachite green, and sulfaguanidine appeared definitely to produce fewer plants showing symptoms of the carnation-mosaic virus. As there was some doubt as to whether the differences in inactivation were significant and produced by the action of the chemical, the second test *in vitro* was replicated and statistically analyzed.

The recording of symptoms in the second replicated experiment, the results of which are illustrated in table 2, was not undertaken until 37 days had elapsed from the time of inoculation. In this case a significant reduction in symptoms was noted at the 5 percent point in all treatments. At the 1 percent point, a significant reduction was noted in all treatments with the exception of proliferol (1 percent) and sulfaguanidine. Two days after inoculation it was noted that all leaves that had been treated with sodium selenate had developed a severe necrosis.

Table 1.--RESULTS OF FIRST CHEMICAL INACTIVATION TEST IN VITRO.

Chemical	Concentration	Number of plants with symptoms[1]
	pct.	plants
Proliferol	10	17
Proliferol	1	3
Sulfamerazine	1	11
Malachite green	1	8
Potassium phosphate	1	14
Potassium chlorate	1	15
Calcium chloride	1	17
Sodium diethyl dithiocarbamate	1	18
Sulfasuxidine	1	12
Trypan blue	1	14
Sulfaguanidine	1	5
Sulfathalidine	1	14
check	-	18

[1] Indicates number of diseased plants out of 24 D. barbatus inoculated. Read after an interval of 25 days from time of inoculation.

Table 2.--ANALYSIS OF RESULTS OF SECOND INACTIVATION TEST IN VITRO.

Chemicals	Concentration	Plants showing symptoms[1]			Average
		Rep. I	Rep. II	Rep. III	
	pct.	plants	plants	plants	plants
Proliferol	10	4	3	2	3.00**
Proliferol	1	5	5	4	4.67*
Malachite green	1	3	1	3	2.33**
Sulfaguanidine	1	4	6	4	4.67*
Sodium selenate	1	1	2	3	2.00**
Zinc sulphate	1	4	3	5	3.00**
Dithane Z78	1	4	3	3	3.33**
Sulfamerizine	1	4	4	4	4.00**
Check	-	7	6	6	6.33

Diff. req. for sig. (odds 19-1) = 1.53*

Diff. req. for sig. (odds 99-1) = 2.12**

[1] Indicates number of D. barbatus plants with symptoms in the three replicates of nine plants each after 37 days.

In vivo tests.--The first chemical inactivation in vivo test was performed during April, May, and June, 1950, when average daily temperatures were gradually rising. These conditions are known to produce masking of the virus (12). This condition was thought to exist in this test. For example, in the flat treated with Dithane Z78 regardless of time of inoculation, the number of plants showing symptoms first increased and then decreased during a period of 16 days as shown in table 3. Because of this and the differing dates of inoculation, symptoms were observed 31 days, 39 days, and 47 days after the first inoculation. The results are recorded in table 3.

In general, treatment of plants with sulfaguanidine, sodium selenate, and proliferol seemed to be more effective in reducing symptoms when applied before inoculations, while Dithane Z78 in this test was more effective when applied after inoculations. Malachite green was uniformly effective in reducing the number of plants expressing symptoms both before and after inoculation, although the number of infected plants appeared to gradually increase. Trypan blue and zinc sulphate were ineffective.

Of the uninoculated checks, only the plants treated with calcium chloride were killed to any extent. This condition was further aggravated in the inoculated groups. Almost one third of the plants inoculated five days after treatment with sodium selenate were killed: however, with the possible exception of the same set treated with sulfaguanidine, none of the other chemicals appeared to be highly lethal.

Table 3.--RESULTS OF FIRST INACTIVATION IN VIVO EXPERIMENT

Chemicals	Concentration	Total chem. applied	Number of plants showing symptoms[1]													
			Inoculated five days before beginning of soil treatments[2]				Inoculated five days after beginning of soil treatments				Inoculated ten days after beginning of soil treatments				Uninoculated check	
			Stand count	31[3] da.	39 da.	47 da.	Stand count	31 da.	39 da.	47 da.	Stand count	31 da.	39 da.	47 da.	Stand count	Maximum Infection
	pct.	gms/sq.ft.														
Calcium chloride	2.0000	106.00	6	1	3	2	1	0	1	1	5	0	3	1	10	0
Sulfaguanidine	0.166	.89	23	10	10	8	19	4	3	3	19	2	5	5	23	0
Zinc sulphate	0.0250	1.33	23	9	7	12	22	6	8	12	19	3	5	9	21	0
Sodium selenate	0.0010	.05	20	4	11	12	15	4	3	4	21	1	1	4	24	0
Proliferol	0.0100	.05	22	9	9	13	20	6	9	14	23	1	3	4	23	0
Malachite green	0.0100	.53	18	4	4	5	24	3	3	5	22	0	0	4	24	0
Trypan blue	0.0100	.53	23	12	13	13	24	3	6	10	22	0	5	7	22	0
Dithane Z78	0.0500	2.67	22	2	6	4	22	4	11	6	21	1	7	5	24	1
Sulfamerizine	0.0166	.89	23	8	6	-[4]	23	9	4	-	24	2	2	-	24	1
p-aminobenzene-sulfonylamide	0.0500	2.67	23	2	9	-[4]	24	0	9	-	19	0	3	-	22	0
Check	--	--	21	9	11	11	24	8	10	8	19	0	5	7	24	0

[1] Total of 24 plants per plot.

[2] Soil treatments applied daily for eight days.

[3] Indicates number of days after first inoculation

[4] No readings taken after 39 days because of confused symptoms due to intense mottling.

About 35 days after soil treatments were begun, the plants treated with p-aminobenzenesulfonylamide and sulfamerizine developed an intense mottling as shown in Fig. 5. This mottling appeared even in the uninoculated check plants and was not typical of that produced by mosaic infection. However, it was sufficiently similar to confuse the mottling produced by mosaic so that readings after 39 days were not taken.

Upon completion of this experiment, the roots of the plants in each flat were washed with running water until all soil was removed. All plant material in each flat, including both roots and tops, was oven dried at 70° C for 72 hours and weighed. These weights are recorded in table 4. Dithane Z78, zinc sulphate, proliferol and sodium selenate appeared to increase the dry weights of plants so treated, while sulfamerizine, sulfaguanidine, and calcium chloride definitely appeared to depress the dry weight.

In the second set of experiments involving inactivation of the virus in vivo, there was no apparent evidence of control in any case. The results are recorded in table 5. Some reduction in symptoms was noted on the carnations treated with calcium chloride, but transmission to D. barbatus revealed evidence of virus activity.

Two separate tests carried on simultaneously, involving the application of chemicals to carnations growing in flats, also indicated no control of the virus. In these tests, the plants were clipped off at ground level after treatments had been applied for 30 days, and were weighed in order to determine any evidence of

Table 4.--OVEN DRY WEIGHTS OF PLANT MATERIAL IN FIRST INACTIVATION IN VIVO TEST.

Chemicals	Concentration	Total chemical applied[1]	Stand count	Total dry weight of plant material[2]	Average dry weight per plant
	pct.	gms./sq. ft	plants	grams	grams
Dithane Z78	0.0500	2.67	89	84.2	.95
Zinc sulphate	0.0250	1.33	85	65.3	.77
Proliferol	0.0100	.05	88	65.5	.74
Sodium selenate	0.0010	.05	80	56.7	.71
Trypan blue	0.0100	.53	91	58.6	.64
CHECK	--	--	88	53.7	.61
p-aminobenenesulfonylamide	0.0500	2.67	88	46.3	.53
Malachite green	0.0100	.53	88	46.1	.53
Calcium chloride	2.0000	106.00	22	9.9	.45
Sulfamerizine	0.0166	.89	94	38.1	.41
Sulfaguanidine	0.0166	.89	84	33.5	.40

[1] Indicates total amount of chemical applied after eight days of application. Applications applied daily.

[2] Oven dry weights of roots and tops of plants. Oven dried at 70°C for 72 hours.

Fig. 4.--Illustration of the effect of p-aminobenzenesulfonylamide on the lower leaves of carnation. (D. caryophyllus). Note die-backs of older basal leaves.

Fig. 5.--Injury on leaves of D. barbatus due to p-aminobenzenesulfonylamide and sulfamerazine. Healthy leaf on left, four injured leaves on right.

Table 5.--RESULTS OF SECOND INACTIVATION TEST IN VIVO.

Chemical	Concentration[1]	Number of infected plants[2]	
		Treated	check
	pct.	plants	plants
Calcium chloride	2.0000	20[3]	20[3]
Sulfaguanidine	0.0166	20	20
Sodium selenate	0.0010	20	20
Zinc sulfate	0.0250	20	20
Proliferol	0.1000	20	20
Malachite green	0.0100	20	20
Trypan blue	0.0100	20	20
Dithane Z78	0.5000	20	20
Sulfamerizine	0.0166	20	20
p-aminobenzene-sulfonylamide	0.0500	20	20

1 Represents concentration in 2 L. of nutrient solution repeatedly applied daily for 14 days to one crock containing two carnation plants (D. caryophyllus).

2 Indicates number of virus-infected plants after treatment for 14 days. Twenty infected plants were utilized in the treatment and check series, respectively.

3 Number of plants infected with virus determined by passing an extract from each D. caryophyllus to four D. barbatus by the abrasion method.

phytotoxicity as measured by a depression in green weight. The results are recorded in table 6. This test indicated that at the concentrations of chemicals used, and for the length of time indicated, there was little or no apparent reduction of green weight of plant material due to treatment. Plant growth was somewhat increased by proliferol and perhaps malachite green.

Effect of scopoletin.--Efforts to inactivate the virus using scopoletin as the inactivating agent proved negative. The results of this test are recorded in table 7. Approximately one third of the 81 D. barbatus treated with a saturated water solution of scopoletin produced virus-like symptoms. These plants were set aside and after a period of approximately 30 days the new growth still had symptoms essentially like those of mosaic. Five of these plants, which seemed to have the most positive mosaic pattern, were tested for virus activity by passage into 20 D. barbatus plants. Four plants were used to test each of the five suspected plants. After an interval of 35 days symptoms of mosaic were not detected on the 20 test plants while symptoms were still evident on the original five plants.

Table 6.--RESULTS OF MODIFIED CHEMICAL INACTIVATION IN VIVO EXPERIMENTS.

Chemical	Concentration	Total weight of chemical applied in 30 days[1]	Average green weight of tops[2]			Number of infected plants[3]	
			Test I	Test II	Ave.	Test I[4]	Test II
	pct.	gms./sq.ft.	grams	grams	grams	plants	plants
Calcium chloride	0.2000	28.00	6.38	5.63	6.01	16	16
Sulfaguanidine	0.0166	2.33	6.38	5.63	6.01	16	16
Zinc sulphate	0.0250	9.50	6.75	6.50	6.63	16	16
Sodium selenate	0.0010	.14	6.69	5.94	6.32	16	16
Proliferol	0.0100	4.20	8.00	7.81	7.91	16	16
Malachite green	0.0010	.14	7.38	6.56	6.97	16	16
Dithane Z78	0.0500	7.00	6.88	6.58	6.73	16	16
Check	--	--	6.33	6.38	6.36	16	16

1 Chemicals and nutrient solution applied at three-fourths strength every other day at the rate of 2.1. of solution per application. Solutions were not applied during cloudy weather.

2 Weights taken 30 days after first application of chemicals.

3 Indicates number of virus-infected carnations (D. caryophyllus) after treatment for 30 days. Sixteen infected plants were utilized in each treatment.

4 Number of plants infected with virus determined by passing an extract from each D. caryophyllus to four D. barbatus by the abrasion technique.

Table 7.--RESULTS OF INACTIVATION TESTS IN VITRO WITH SCOPOLETIN.

Light treatment[1]		Number of plants showing symptoms[2]		
72 hours before inoculation	72 hours after inoculation	Virus[3]	Scopoletin[4]	Virus and scopoletin[5]
Exposed	Exposed	25	8	24
Exposed	Dark	24	9	22
Dark	Exposed	22	9	22

1 Indicates light exposure for 72 hours before and after inoculation.

2 Indicates number of plants manifesting symptoms out of 27 plants in each treatment.

3 Virus in infectious centrifuged plant juice.

4 Saturated solution of scopoletin in distilled water (1:4000).

5 Saturated solution of scopoletin in infectious centrifuged plant juice.

Chapter VI

DISCUSSION

Tests on the effect of proliferol, malachite green, sulfaguanidine, sulfamerizine, sodium selenate, zinc sulphate, and Dithane Z78 on carnation mosaic virus indicated some apparent loss of infectivity when applied *in vitro*. However, facilities were not available for removing the chemicals after their application to the virus. Accordingly, the action of the chemical not only on the virus, but also upon the plant involved in the inoculation, must be considered. The possible masking of symptoms due to absorbtion of the chemical by the plant and the effects of the chemical on the plant cells could possibly have influenced the manifestation of mosaic infection.

The progress of the virus in a plant is generally considered to be by the invasion of living cells and to be hindered by death of the cells. Therefore, a chemical which is toxic to the cell might hamper or stop the movement of the virus and thereby limit infection. Obvious evidence of this was noted in the use of sodium selenate, which produced a severe necrosis of the inoculated leaf. Fewer plants of *D. barbatus*, thus treated, appeared to express symptoms of virus infection.

From the evidence presented there seems to be little possibility of the carnation-mosaic virus being inactivated *in vivo* by the chemicals used in these experiments after the virus has become established in a plant. However, the treatment of plants with chemicals

before infection by the virus occurs might meet with some success. Stoddard (23) has postulated that chemicals which are effective in the control of a virus before inoculation have an effect on the plant which prevents multiplication of the virus and that chemicals effective after inoculation have a direct action on the virus itself after it is present in the plant. As Dithane Z78 applied after inoculation was more effective in reducing the number of plants showing symptoms, it may be possible that its action was directly on the virus in the plant. In contrast, the action of sulfaguanidine, sodium selenate, and proliferol may be on the plant, thereby possibly producing immunity to infection by the virus. However, due to the necrotic condition of leaf tissue treated with such a chemical as sodium selenate, it may be postulated that the presence of a toxic compound within a cell may prevent its recovery after wounding to such an extent that the virus is unable to produce infection.

Malachite green, while effective both before and after inoculation, permitted a gradual increase in expression of symptoms. According to Takahashi (25), this chemical produces a reduction in the increase of tobacco-mosaic virus. This may be an explanation of the failure of symptoms to show for some time and later gradually to appear. This condition would be further aggravated by the tendency of the virus to become masked due to rising temperatures.

It appears from these experiments that the chemicals with the most promise of inactivating the virus have very little

phytotoxic effect and in some cases increase growth either due to stimulation or by providing nutrients to soils lacking these elements.

Scopoletin does not appear to be an inactivant of the carnation-mosaic virus *in vitro*. The mottling symptoms observed on the plants inoculated with only a water solution of scopoletin was not a result of virus activity, as proved by unsuccessful transmission attempts. Best (4) reported an increased amount of scopoletin in tobacco leaves as a result of infection with tomato spotted-wilt virus. In view of these findings it may be postulated that the presence of scopoletin in a leaf may be in part the cause of symptoms due to virus activity.

Suggestions for future research.

1. Emphasis should be placed on prevention of infection by the virus rather than curing a plant already infected.

2. Screening of new chemicals should be undertaken on D. barbatus rather than D. caryophyllus due to the difficulty of reading symptoms on the latter.

3. The experiment involving the application of chemicals before and after inoculation should be repeated using the more promising chemicals. These include Dithane Z78, Proliferol, malachite green, and sodium selenate. All plants should be carefully screened for virus content by means of passage to healthy plants.

4. For more critical study, a method should be devised whereby fluctuations in the virus content of the host can be detected along with absolute diagnosis as to whether the virus is present or not. Perhaps electrophoretic detection of the plant virus proteins could be utilized.

5. A critical study of the factors influencing masking of symptoms.

Chapter V

SUMMARY

The influence of various chemicals on the infectivity of the carnation mosaic virus has been studied both *in vitro* and *in vivo*.

Preliminary tests indicated a loss of infectivity in transmission of the virus by treatment *in vitro* with Proliferol, malachite green, and sulfaguanidine. In addition further experiments showed a loss in infectivity of the virus when treated with sodium selenate, zinc sulphate, Dithane Z78, and sulfamerizine.

The application of chemicals by soil watering has been made to *Dianthus barbatus* both before and after inoculation. Treatment of plants with sulfaguanidine, sodium selenate, and proliferol appeared to be more effective in reducing symptoms of the virus when applied before inoculations, while Dithane Z78 was more effective when applied after inoculations. Malachite green was uniformly effective in reducing the number of plants expressing symptoms both before and after inoculation.

Efforts to inactivate the virus in carnation plants (*D. caryophyllus*) already infected with the virus by means of chemicals dissolved in nutrient solutions proved unsuccessful.

Chemicals with the most promise of inactivating the virus have very little phytotoxic effect and in some cases increase growth. Applications of p-aminobenzenesulfonylamide and sulfamerizine produced an intense mottling on *D. barbatus* and a dieback of the leaves

of *D. caryophyllus*.

Scopoletin does not inactivate the carnation-mosaic virus *in vitro*. When, however, a saturated water solution of Scopoletin was applied by the carburundum technique, a mottling resulted very similar in appearance to the carnation-mosaic virus. It is postulated that the presence of scopoletin in a leaf may be in part the cause of symptoms due to virus activity.

It is believed that some evidence is available which indicates the presence of a toxic compound within a cell may prevent its recovery after wounding to such an extent that an introduced virus is unable to produce infection.

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